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(54) **RECOMBINANT EXPRESSION VECTOR SYSTEM FOR VARIANTS OF COAGULATION FACTOR VIII AND VON WILLEBRAND FACTOR**

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(30) **Foreign Application Priority Data**

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C07K 14/755 (2006.01)

(52) **U.S. Cl.**
CPC ***C07K 14/755*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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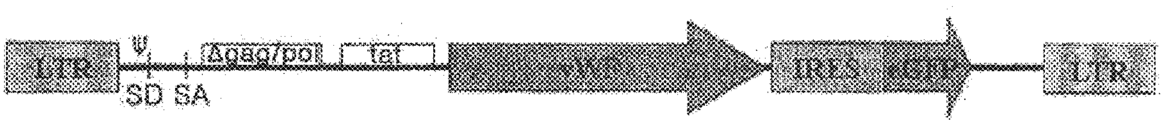
(57) **ABSTRACT**

Disclosed is an expression vector system for variants of coagulation Factor VIII (FVIII) and von Willebrand Factor (vWF). In detail, mutant vWF the size of which is significantly reduced by deleting exons but which has remarkably increased FVIII stabilizing and activating efficiency, and an expression vector system useful for the treatment of hemophilia which is capable of expressing the same along with FVIII are disclosed. Use of the mutant vWF with a reduced size enables effective expression of FVIII in a viral vector and significantly enhanced FVIII activity. Further, the viral vector may be effectively used to treat hemophilia through gene therapy.

3 Claims, 14 Drawing Sheets



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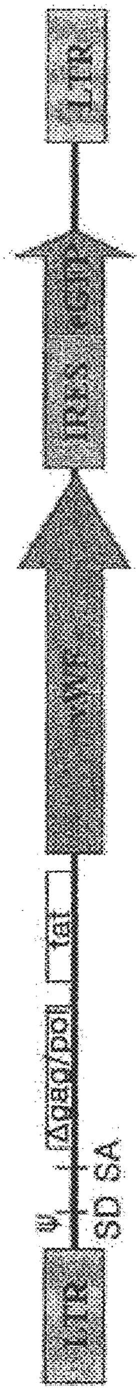


FIGURE 1

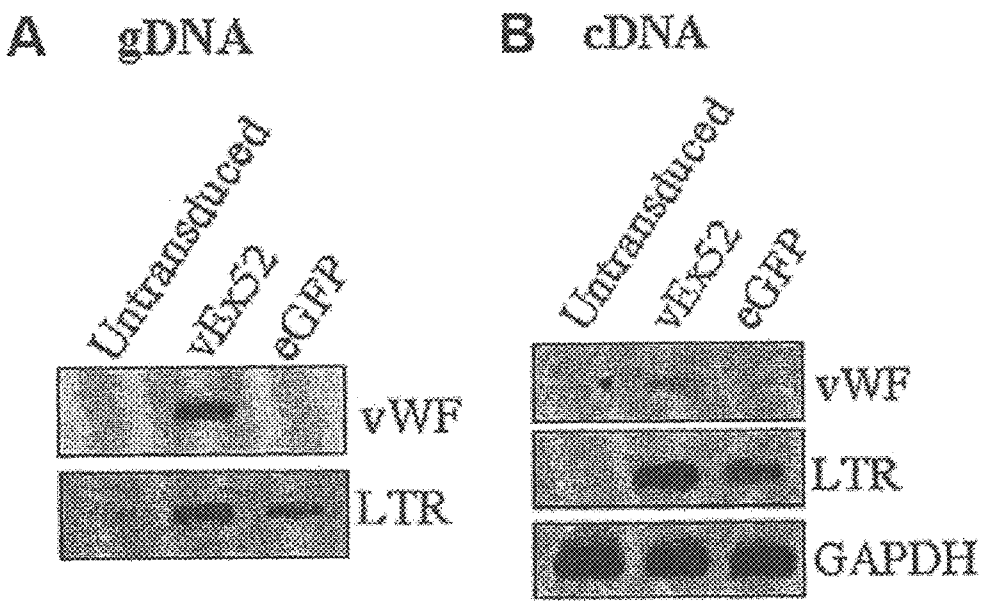

A gDNA
Untransduced
vEx52
eGFP
vWF
LTR
B cDNA
Untransduced
vEx52
eGFP
vWF
LTR
GAPDH

FIGURE 2

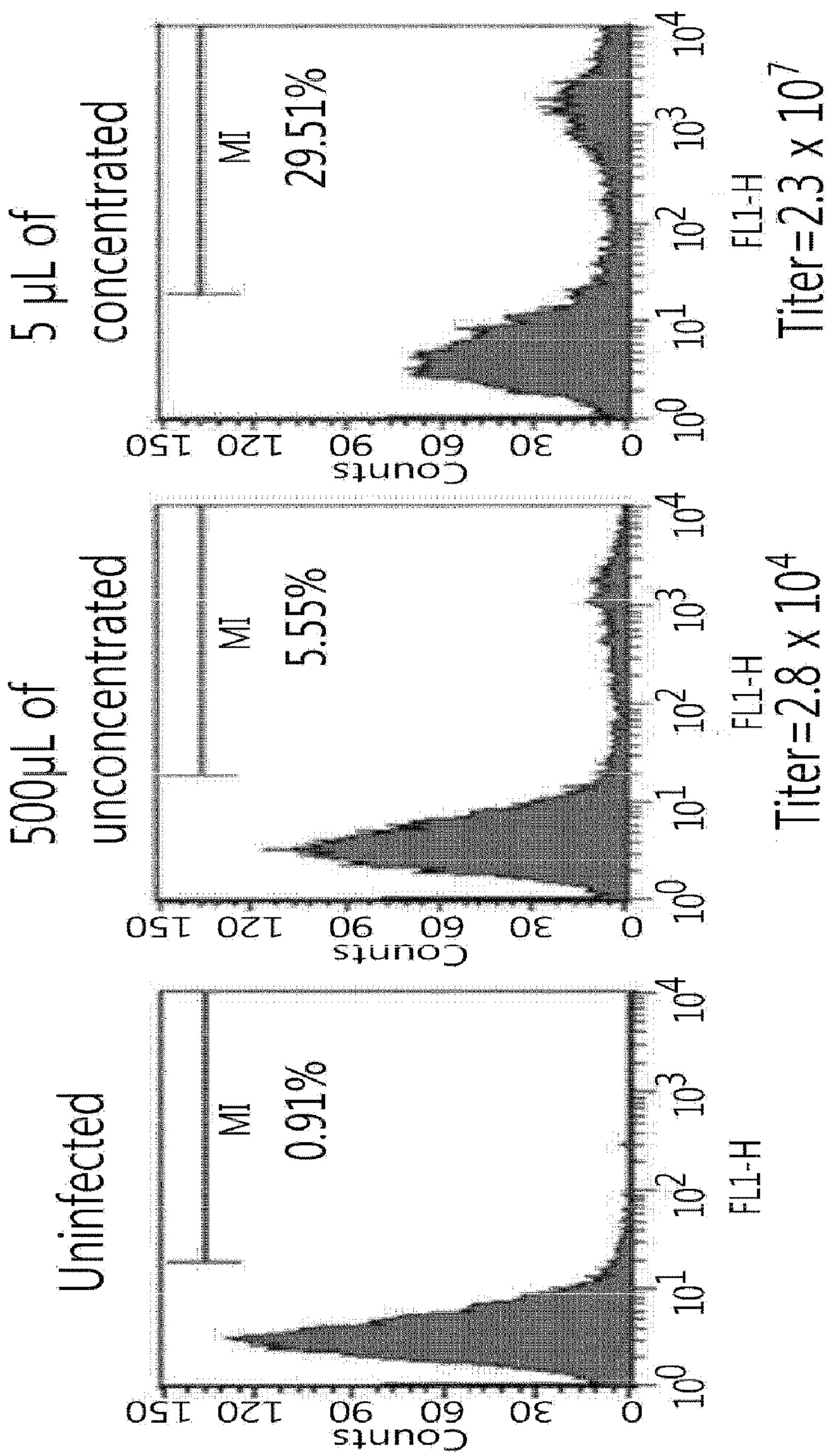

Uninfected
MI
0.91%
Counts
FL1-H
500μL of
unconcentrated
MI
5.55%
Counts
FL1-H
Titer=2.8 x 10^4
5 μL of
concentrated
MI
29.51%
Counts
FL1-H
Titer=2.3 x 10^7

FIG. 3

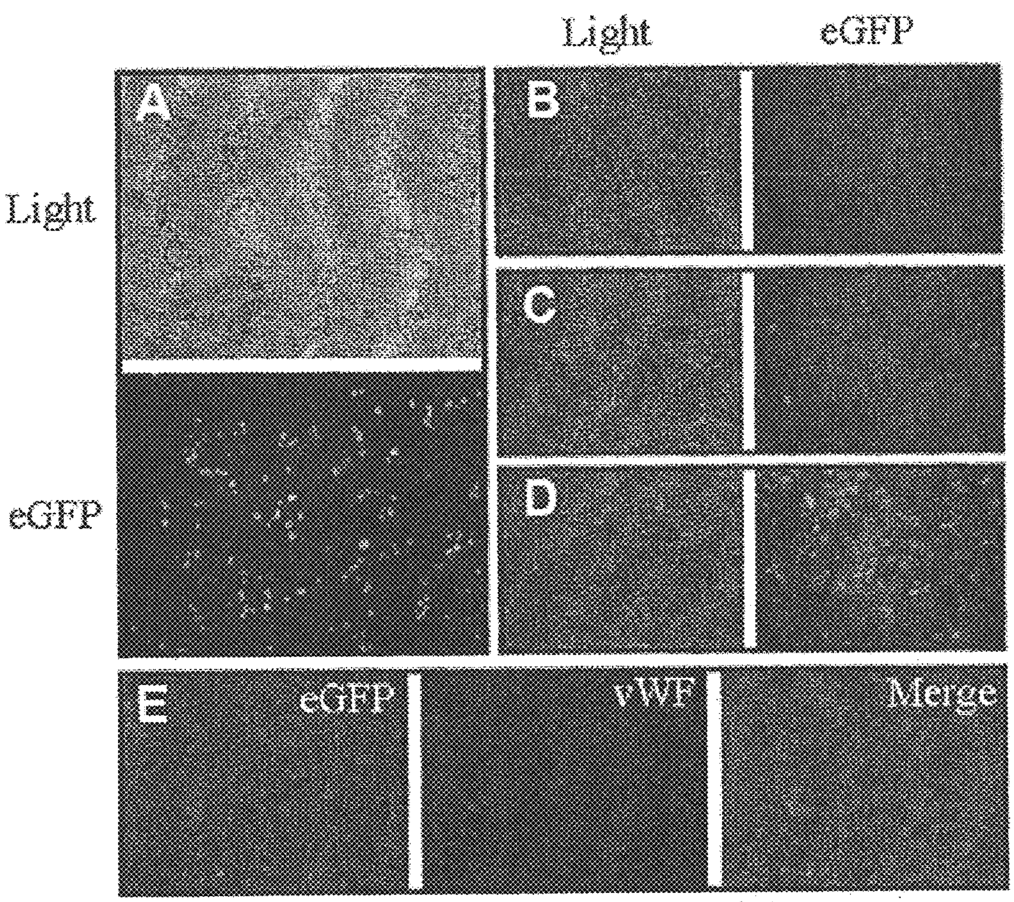


FIGURE 4

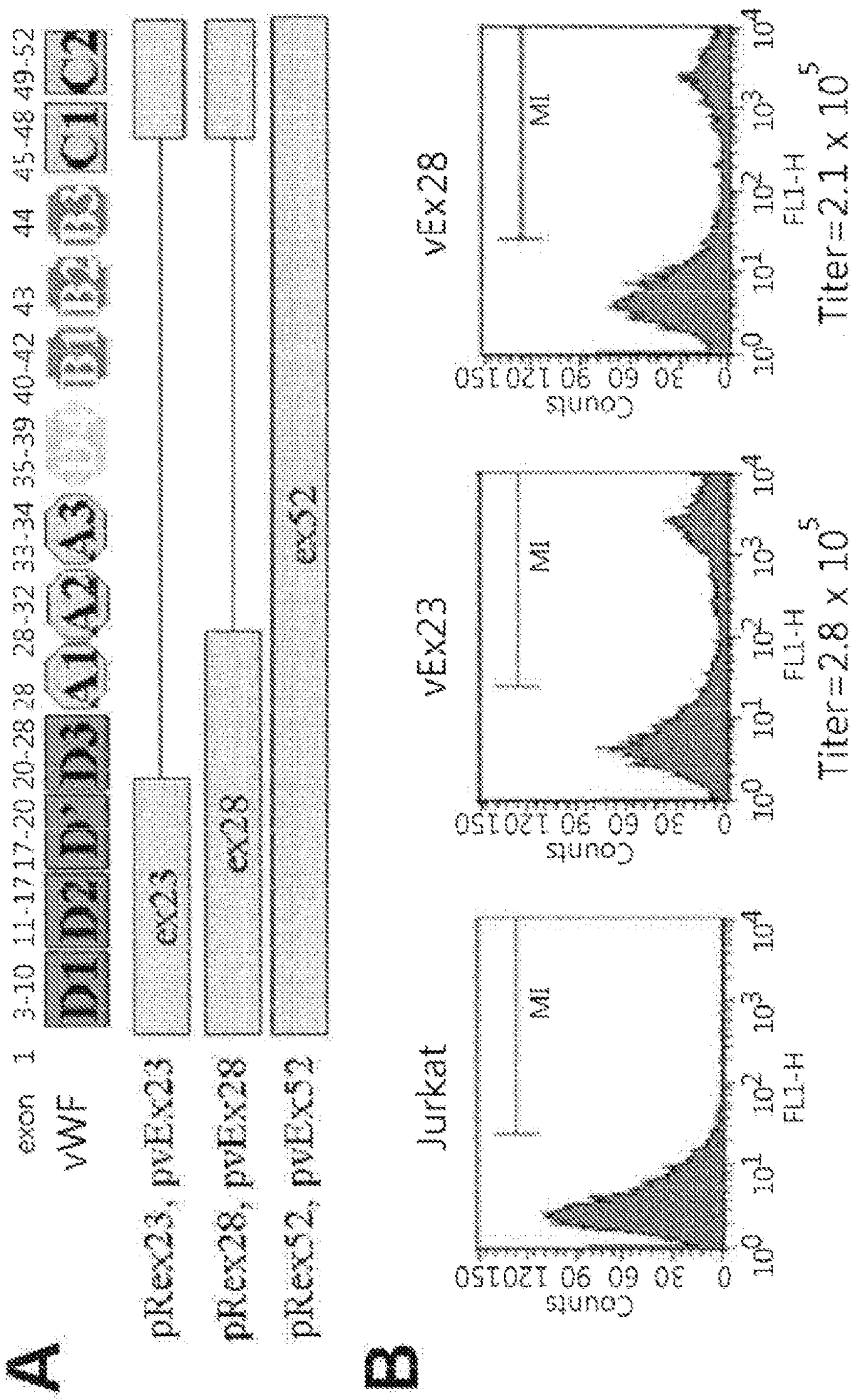

A
B
exon
vWF
pRex23, pvEx23
pRex28, pvEx28
pRex52, pvEx52
ex23
ex28
ex52
Jurkat
vEx23
vEx28
MI
Counts
FL1-H
Titer=2.8 x 10^5
Titer=2.1 x 10^5

FIG. 5

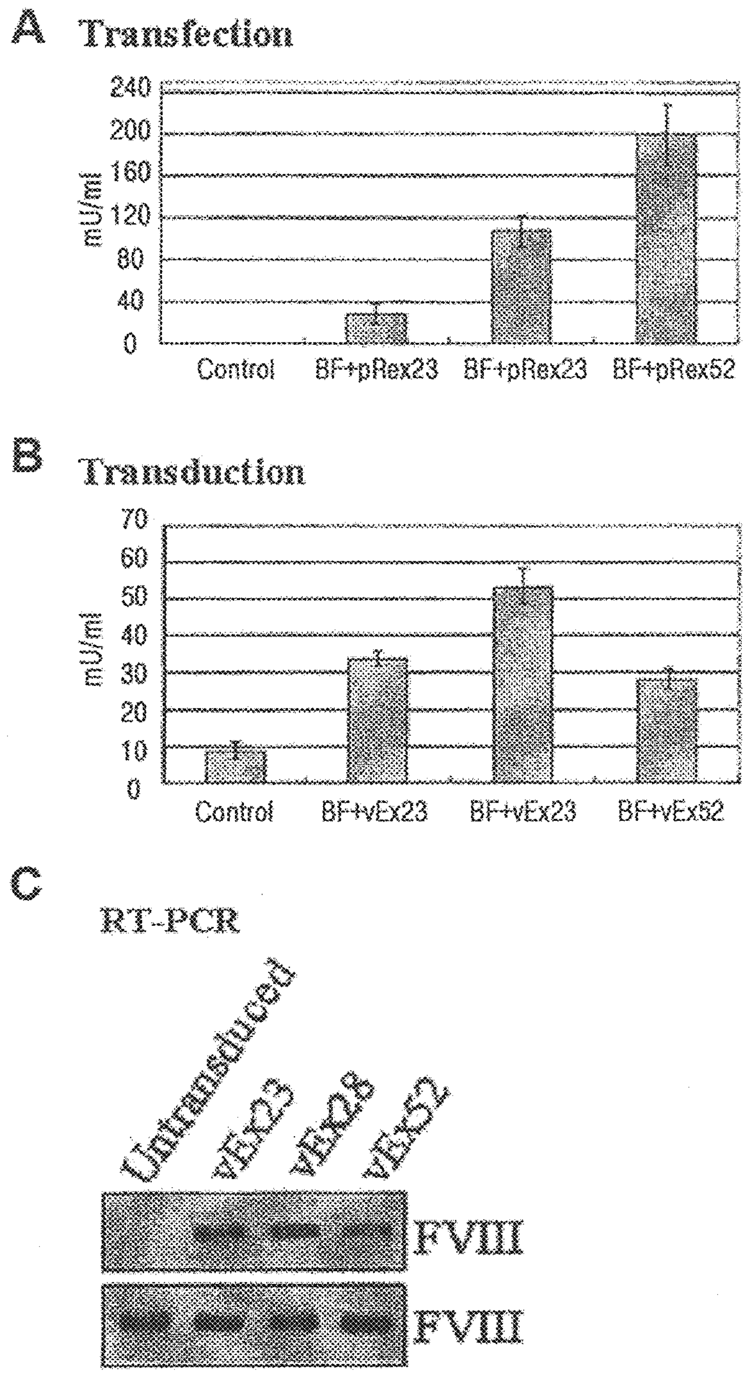

A
Transfection
240
200
160
120
80
40
0
mU/ml
Control
BF+pRex23
BF+pRex23
BF+pRex52
B
Transduction
70
60
50
40
30
20
10
0
mU/ml
Control
BF+vEx23
BF+vEx23
BF+vEx52
C
RT-PCR
Untransduced
vEx23
vEx28
vEx52
FVIII
FVIII

FIGURE 6

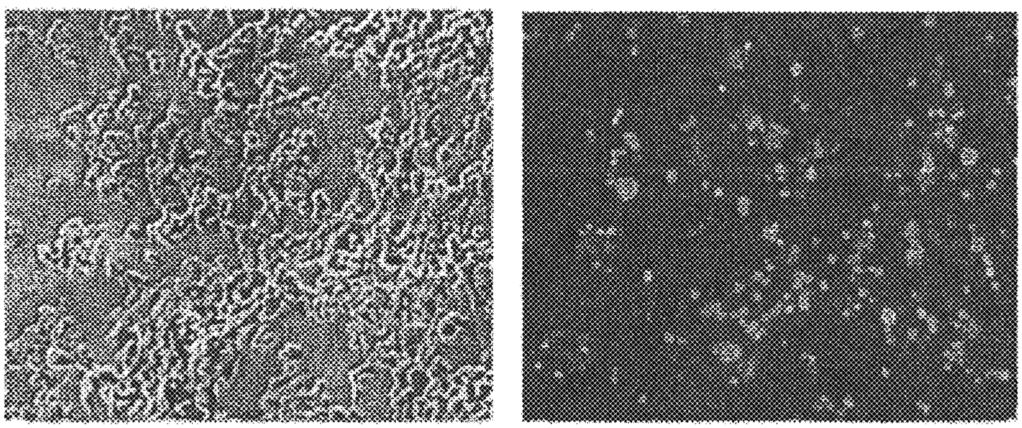


FIGURE 7

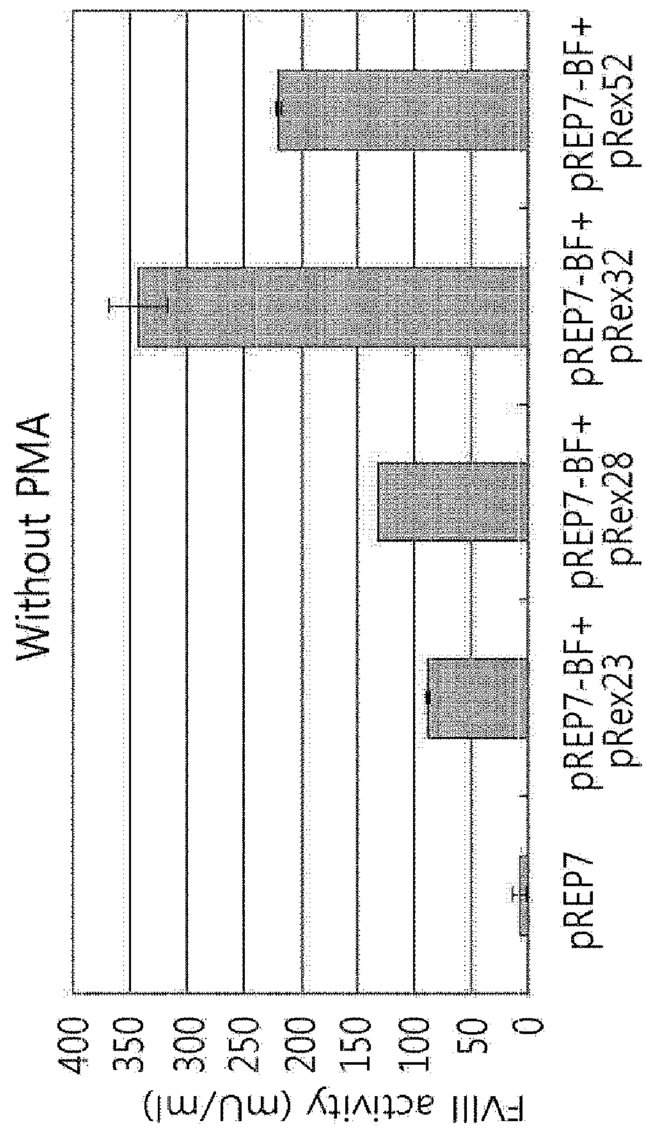

Without PMA
FVIII activity (mU/ml)
400
350
300
250
200
150
100
50
0
pREP7
pREP7-BF+ pRex23
pREP7-BF+ pRex28
pREP7-BF+ pRex32
pREP7-BF+ pRex52

FIG. 8A

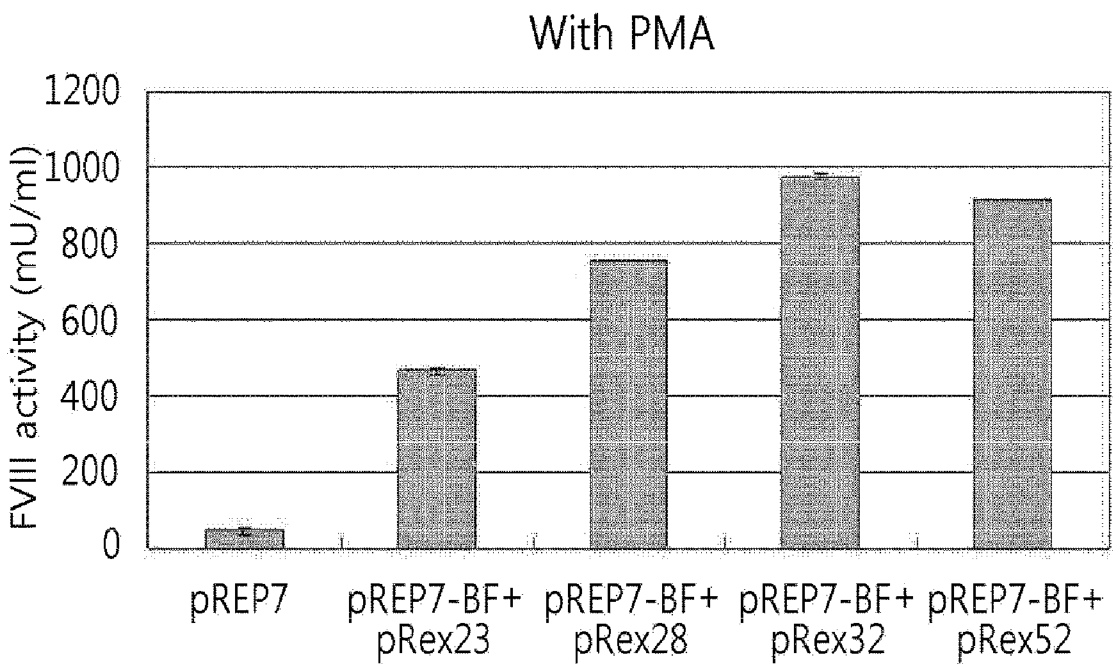

With PMA
FVIII activity (mU/ml)
1200
1000
800
600
400
200
0
pREP7
pREP7-BF+ pRex23
pREP7-BF+ pRex28
pREP7-BF+ pRex32
pREP7-BF+ pRex52

FIG. 8B

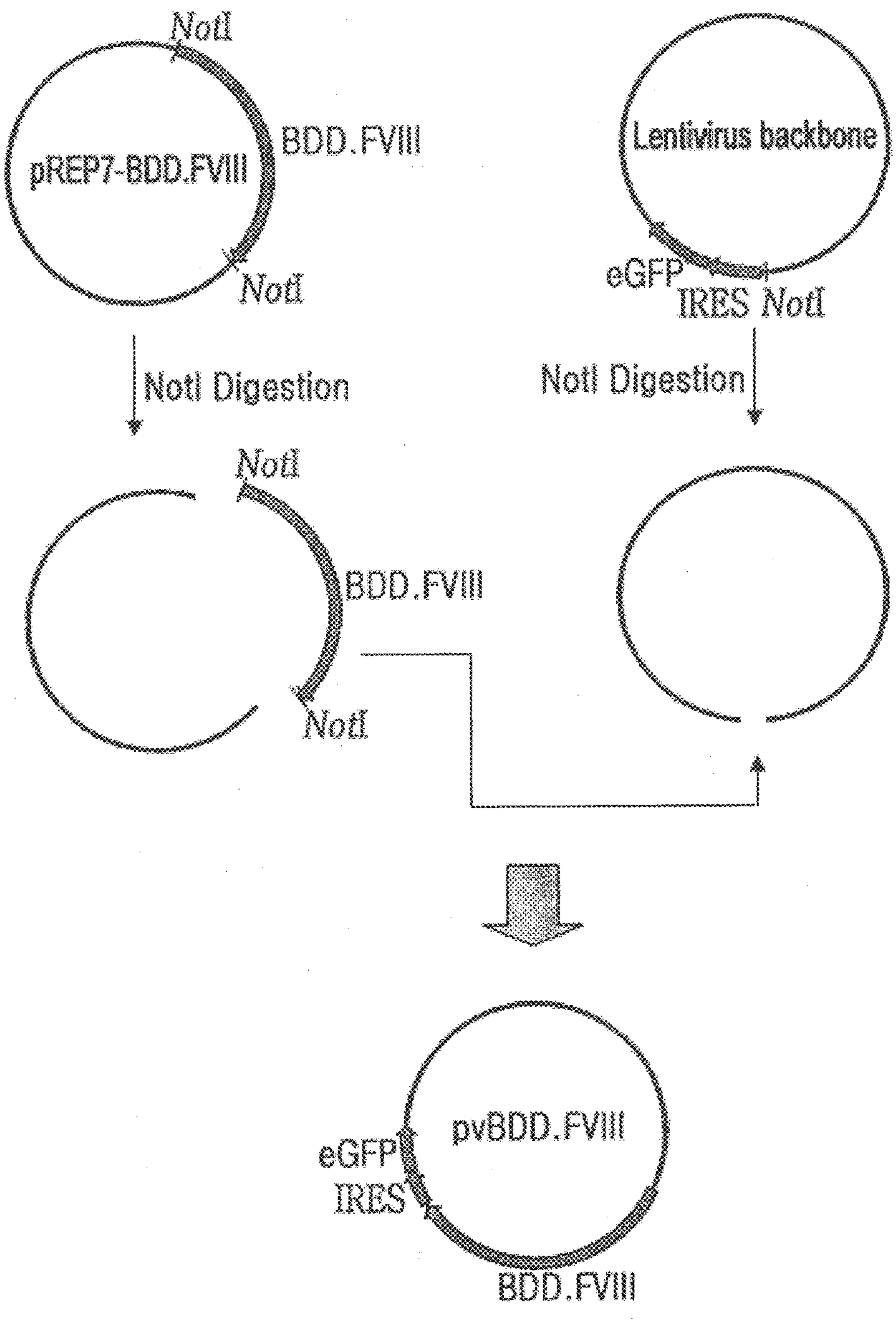

NotI
pREP7-BDD.FVIII
BDD.FVIII
NotI
Lentivirus backbone
eGFP
IRES NotI
NotI Digestion
NotI Digestion
NotI
BDD.FVIII
NotI
pvBDD.FVIII
eGFP
IRES
BDD.FVIII

FIGURE 9

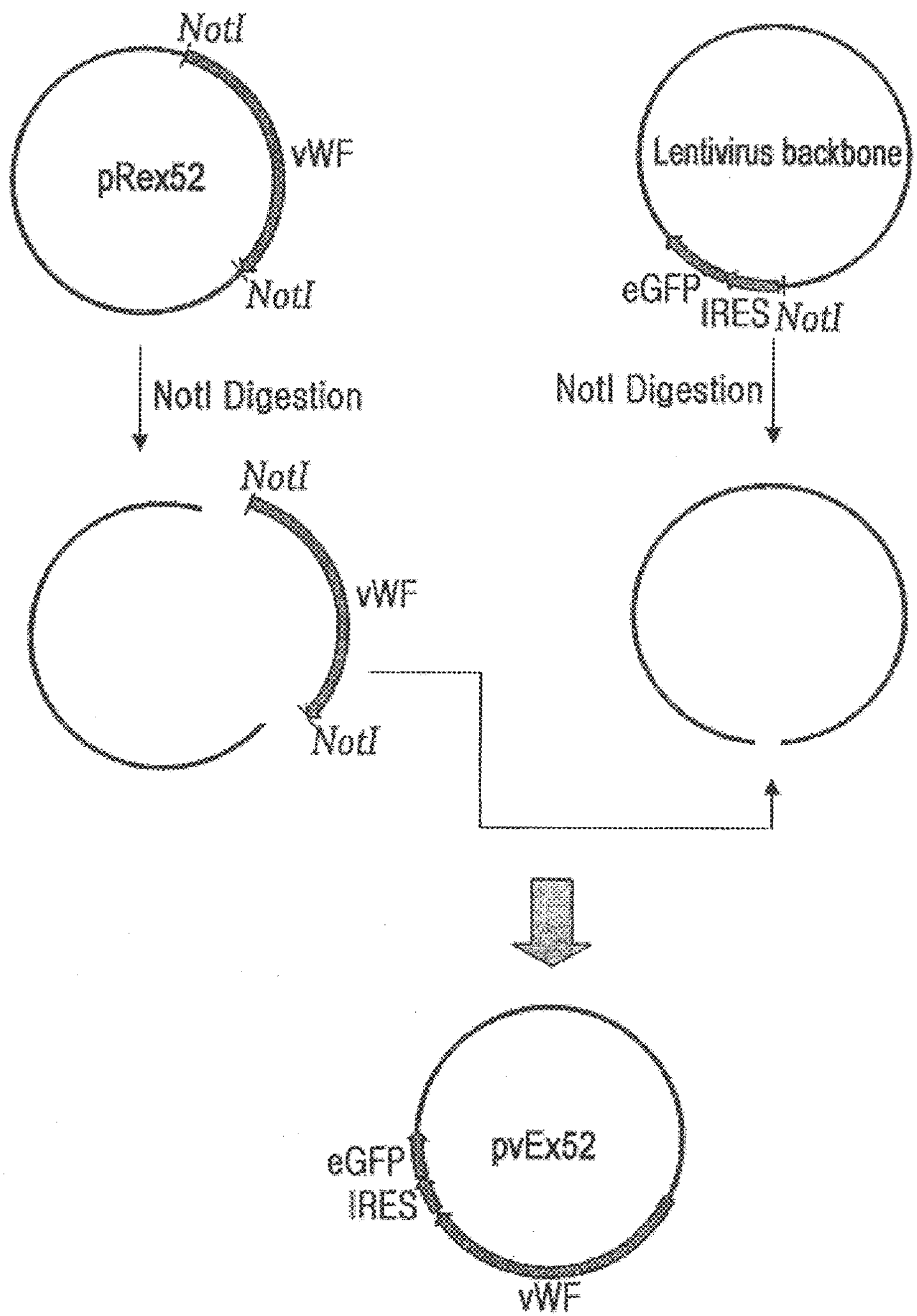
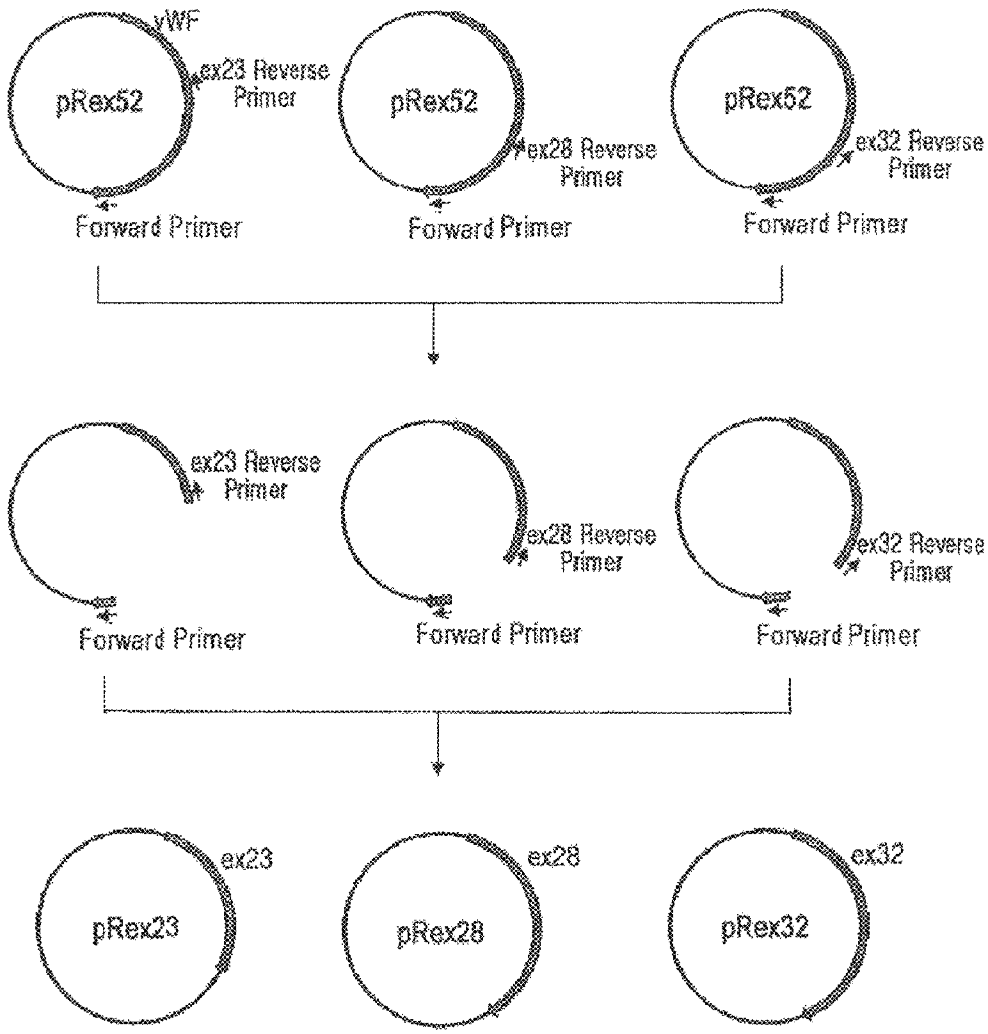

NotI
pRex52
vWF
NotI
Lentivirus backbone
eGFP
IRES
NotI
NotI Digestion
NotI Digestion
NotI
vWF
NotI
pvEx52
eGFP
IRES
vWF

FIGURE 10



vWF
pRex52
ex23 Reverse Primer
Forward Primer
pRex52
ex28 Reverse Primer
Forward Primer
pRex52
ex32 Reverse Primer
Forward Primer
ex23 Reverse Primer
Forward Primer
ex28 Reverse Primer
Forward Primer
ex32 Reverse Primer
Forward Primer
ex23
pRex23
ex28
pRex28
ex32
pRex32

FIGURE 11

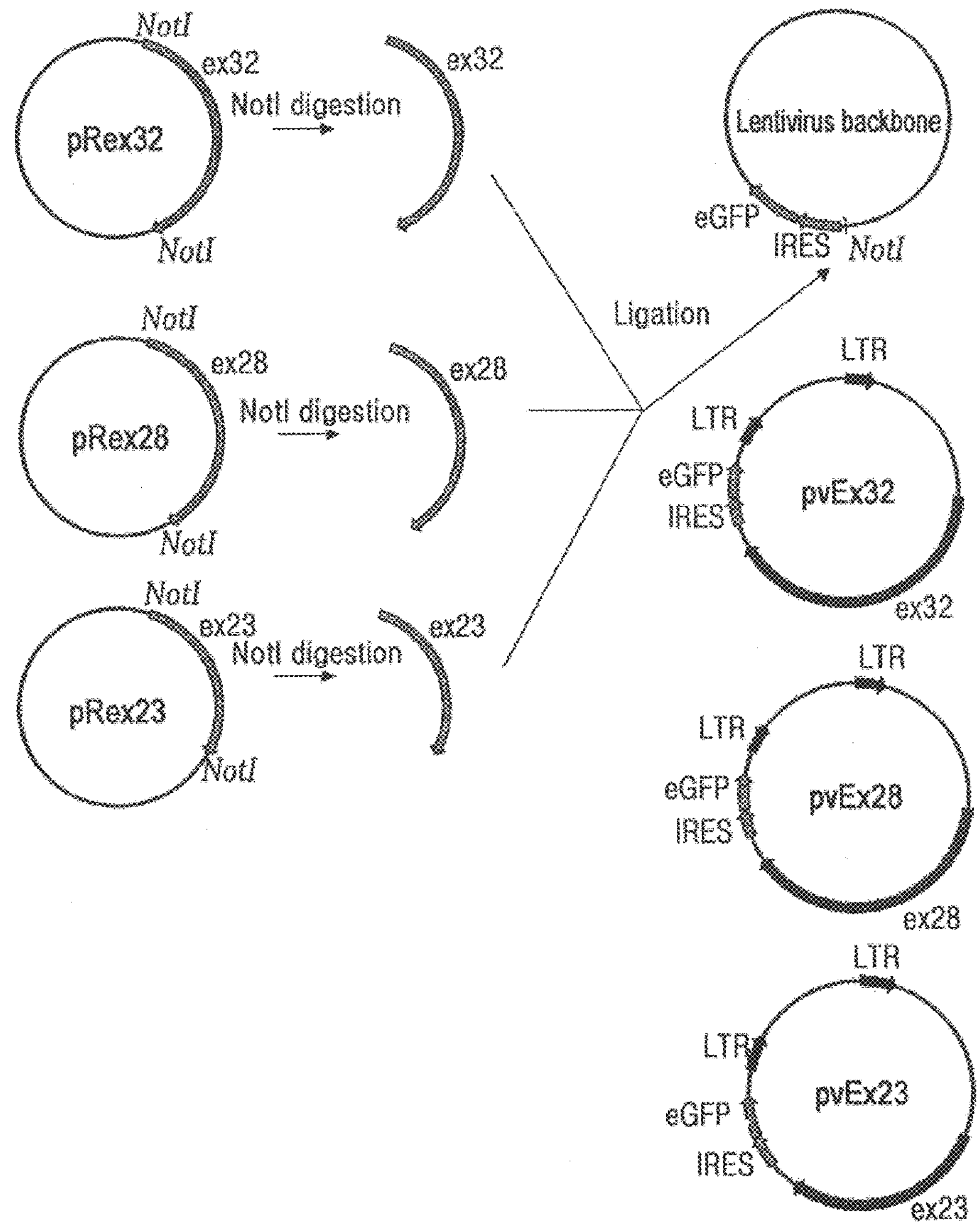

NotI
ex32
NotI digestion
pRex32
NotI
ex32
Lentivirus backbone
eGFP
IRES
NotI
Ligation
NotI
ex28
NotI digestion
pRex28
NotI
ex28
LTR
LTR
eGFP
IRES
pvEx32
ex32
NotI
ex23
NotI digestion
pRex23
NotI
ex23
LTR
LTR
eGFP
IRES
pvEx28
ex28
LTR
LTR
eGFP
IRES
pvEx23
ex23

FIGURE 12

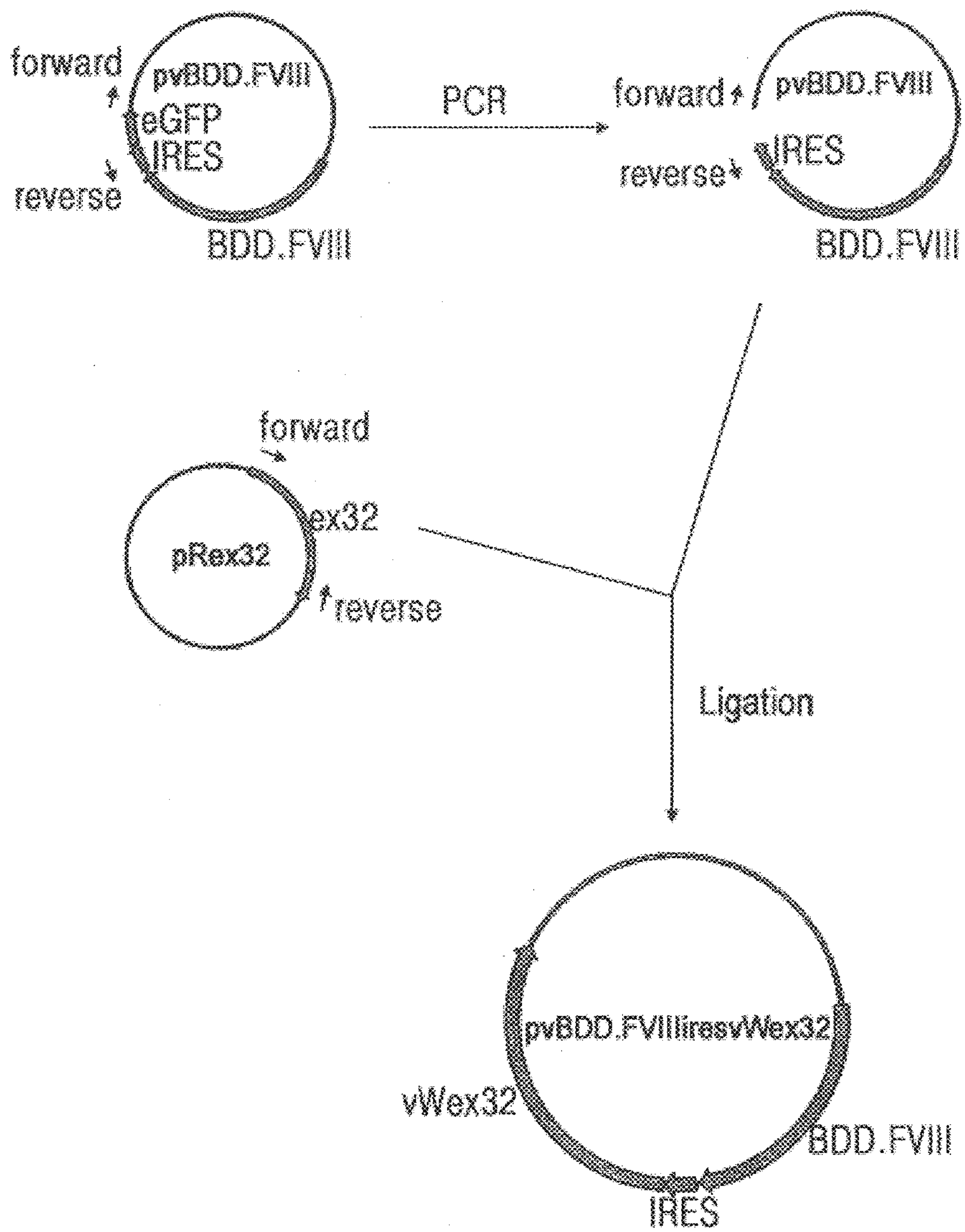

forward
pvBDD.FVIII
eGFP
IRES
reverse
BDD.FVIII
PCR
forward
pvBDD.FVIII
IRES
reverse
BDD.FVIII
forward
ex32
pRex32
reverse
Ligation
pvBDD.FVIIIiresvWex32
vWex32
BDD.FVIII
IRES

FIGURE 13

RECOMBINANT EXPRESSION VECTOR SYSTEM FOR VARIANTS OF COAGULATION FACTOR VIII AND VON WILLEBRAND FACTOR

CROSS-REFERENCE TO RELATED APPLICATION

This application is a division of U.S. patent application Ser. No. 12/200,928 filed on Aug. 28, 2008. U.S. Ser. No. 12/200, 928 claims the benefit of Korean Patent Application No. 10-20080019392, filed on Feb. 29, 2008, in the Korean Intellectual Property Office. The disclosures of which are incorporated herein in their entirety by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 30, 2014, is named 88315DIV50498_ST25.txt and is 84,678 bytes in size.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an expression vector system for variants of coagulation Factor VIII (FVIII) and von Willebrand Factor (vWF), more particularly to mutant vWF the size of which is significantly reduced by deleting exons but which has remarkably increased FVIII stabilizing and activating efficiency, and an expression vector system useful for the treatment of hemophilia which is capable of expressing the same along with FVIII.

2. Description of the Related Art

Hemophilia A is a hereditary, X chromosome-linked blood clotting disorder caused by a deficiency in FVIII. Symptoms include frequent bleeding in muscles, bones, digestive and urinary tracts, etc. accompanied by swelling and pain. Current treatment is based on regular supplementation of FVIII. This requires a lifelong treatment, giving troubles in daily lives and economic burdens. Further, there is a high risk of secondary infection during its administration.

FVIII is a large glycoprotein of 180 Kb, and consists of A1-A2-B-A3-C1-C2 domains. The FVIII gene is located on the X chromosome, and its synthesis is carried out mostly in the liver. Until now, there have been a lot of researches to transducer FVIII, but there were many difficulties because its size was too large, or the transduced FVIII gene was not expressed or secreted well. The B domain of FVIII consists of a large exon and its asparagine, serine and threonine residues are highly glycosylated. According to recent functional studies, the domain is not essential in procoagulant activity, and the deletion thereof does not affect the function of FVIII. When B-domain deleted FVIII (BDD-FVIII) was expressed in cells, the problems of unstable FVIII mRNA structure and interaction with ER chaperones were overcome and a lot of FVIII mRNA could be attained. Of the BDD-FVIII, a variant with 226 amino acids at the N-terminal with 6 consensus site for N-linked glycosylation exhibited significantly increased FVIII secretion.

In genetic treatment of hemophilia A, the target cell is bone marrow cells, especially stem cells or progenitor cells. Lentivirus-based vectors are used to transfer the gene. After infection into cells, these vectors insert the gene into the chromosome of the infected cell, thereby enabling stable and consistent expression. Other viruses such as Moloney murine leukemia virus could not be used to infect stem cells or progenitor cells, because they infect only dividing cells. And, although adenovirus produces a large amount of expressed proteins, a consistent expression was impossible because the gene is diluted as the differentiation continues.

Accordingly, a safe and consistent way of transducing FVIII is necessary, and the development thereof is needed. Lentiviral vectors can infect nearly all non-dividing cells, as well as dividing cells, and provide stable expression for a long period of time because they are inserted in the cell chromosome after the infection. Thus, lentivirus-based vectors for expression of FVIII may be useful for gene therapy.

vWF plays an important role in activating FVIII during blood coagulation. vWF is a blood glycoprotein which binds to FVIII thereby preventing it from being degraded in the blood. Besides, it plays a major role in blood coagulation by binding to collagen or platelet when endothelial cells are injured. vWF consists of D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2 domains, and the D'-D3 domain binds to FVIII. vWF is a 250 kDa-sized protein and its gene is about 9 Kb in size. Accordingly, it is impossible to insert vWF in a lentiviral vector to help the function of FVIII. Through researches on the essential part in the vWF domains with respect to activation of FVIII, the inventors of the present invention found out that the portion of the vWF gene up to exon 32 functions most efficiently. Based on this finding, we inserted FVIII, an internal ribosome entry site (IRES) and vWF in a lentivirus-based vector. The resultant viral vector expresses the proteins gag-pol, env, tat and rev required for lentivirus, thereby expressing FVIII and vWF upon infection of cells. This attempt has never been made and is valued very highly for gene therapies and hemophilia researches in the future.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to provide mutant von Willebrand Factor (vWF) the size of which is significantly reduced by deleting exons but which has remarkably increased coagulation Factor VIII (FVIII) stabilizing and activating efficiency.

The present invention is also directed to provide a vector which expresses FVIII and vWF consistently and stably in cells.

Through expression of the factors, the present invention aims at providing a successful gene therapy for hemophilia A. The present invention is distinguished from other existing inventions in that not only FVIII but also vWF, which is essential for the function thereof, is expressed together.

To prove the effect of the present invention, VSV-G pseudotyped lentivirus expressing FVIII and vWF was produced using a lentivirus-based vector and transfected into various cells. Then, the activity of FVIII expressed in the cells was measured. It was determined by quantitating the level of activation of Factor X by FVIII and activated Factor IX.

In an aspect, the present invention provides mutant vWF (vWF23) having an amino acid sequence of SEQ ID NO: 2 in which exons 24-46 of vWF are deleted.

In another aspect, the present invention provides a mutant vWF23 gene having a base sequence encoding for a protein having an amino acid sequence of SEQ ID NO: 2. Preferably, the gene may have a base sequence of SEQ ID NO: 1.

In another aspect, the present invention provides mutant vWF (vWF28) having an amino acid sequence of SEQ ID NO: 4 in which exons 29-46 of vWF are deleted.

In another aspect, the present invention provides a mutant vWF28 gene having a base sequence encoding for a protein having an amino acid sequence of SEQ ID NO: 4. Preferably, the gene may have a base sequence of SEQ ID NO: 3.

In another aspect, the present invention provides mutant vWF (vWF32) having an amino acid sequence of SEQ ID NO: 6 in which exons 33-48 of vWF are deleted.

In another aspect, the present invention provides a mutant vWF32 gene having a base sequence encoding for a protein having an amino acid sequence of SEQ ID NO: 6. Preferably, the gene may have a base sequence of SEQ ID NO: 5.

In another aspect, the present invention provides an animal cell expression vector comprising a gene encoding for the mutant vWF (vWF23, vWF28 or vWF32).

In the present invention, the animal cell expression vector may be any non-viral (plasmid or liposome) or viral vector capable of delivering and expressing the gene in an animal cell. Preferably, it may be a viral vector such as retroviral vector, lentiviral vector, adenoviral vector and adeno-associated viral vector. More preferably, it may be a lentiviral vector. In FIG. **12**, lentiviral vectors pvEx23, pvEx28 and pvEx32, which express the vWF23, vWF28 and vWF32, respectively, are disclosed.

In the present invention, the animal cell expression vector may further comprise a gene encoding for B-domain-deleted human FVIII. In this case, the two effective ingredients for the treatment of hemophilia may be expressed using a single vector.

In the present invention, the B-domain-deleted human FVIII may preferably have an amino acid sequence of SEQ ID NO: 8, and its gene may have a base sequence of SEQ ID NO: 7.

In the present invention, the animal cell expression vector capable of expressing both the mutant vWF (vWF23, vWF28 or vWF32) and the B-domain-deleted human FVIII may be any non-viral (plasmid or liposome) or viral vector. Preferably, it may be a viral vector such as retroviral vector, lentiviral vector, adenoviral vector and adeno-associated viral vector. More preferably, it may be a lentiviral vector. In FIG. **13**, a pvBDD.FVIII.ires.vWex32 lentiviral vector in which the two genes are linked by an internal ribosome entry site (IRES) is disclosed as a bicistronic expression system.

In another aspect, the present invention provides lentiviral particles packaged by transfecting the lentiviral vector capable of expressing the mutant vWF or B-domain-deleted human FVIII in a packaging cell.

In the present invention, the packaging cell may be any one capable of packaging the lentiviral vector to form lentiviral particles, such as 293T cells and HT1080 cells. Preferably, 293T cells may be used.

In the present invention, the lentiviral vector is cotransfected with pGag-pol, pRev, pTat and pVSV-G in order to form the lentiviral particles. In the example that follows, a split gene expression system was used for safe production of viruses. That is, only the factors gag-pol, tat, rev and VSV-G essential for the production of viruses were expressed, but they were delivered through different vectors in order to reduce the possibility of recombination.

In another aspect, the present invention provides a pharmaceutical composition for the treatment and prevention of hemophilia comprising the animal cell expression vector or the mutant vWF and B-domain-deleted human FVIII expressed therefrom as an active ingredient.

In another aspect, the present invention provides a pharmaceutical composition for the treatment and prevention of hemophilia comprising the lentiviral particles as an active ingredient.

Hereinafter, the present invention will be described in more detail.

The inventors of the present invention developed a lentiviral-based expression vector system which expresses coagulation Factor VIII (FVIII) and mutant von Willebrand factor (vWF) at the same time. Specifically, we confirmed the expression and activation of FVIII by the lentiviral-based system and elucidated the domain of vWF essential for the activation of FVIII.

The FVIII use in the present invention is a B-domain-deleted FVIII (BDD-FVIII) for increasing the secretion of FVIII. The B domain of FVIII consists of one large exon and its asparagine, serine and threonine residues are highly glycosylated. According to recent functional studies, the domain is not essential in procoagulant activity, and the deletion thereof does not affect the function of FVIII. When BDD-FVIII was expressed in cells, the problems of unstable FVIII mRNA structure and interaction with ER chaperones were overcome and a lot of FVIII mRNA could be attained.

Of the BDD-FVIII, a variant with 226 amino acids at the N-terminal with 6 consensus site for N-linked glycosylation exhibited significantly increased FVIII secretion. Lentiviral vectors can infect nearly all non-dividing cells, as well as dividing cells, and provide stable expression for a long period of time because they are inserted in the cell chromosome after the infection. Thus, lentivirus-based vectors for expression of FVIII may be useful for gene therapy.

The mutant vWF of the present invention has some of its entire exons deleted, and comprises only up to D1-D2-D'-D3 domains (vWF23), D1-D2-D'-D3-A1 domains (vWF28), or D1-D2-D'-D3-A1-A2 domains (vWF32). These domains bind to FVIII, to FVIII and platelet GP1 b, or to FVIII, platelet GP1 b and collagen, respectively.

In order to maximize the FVIII activity, expression of vWF is required. It protects FVIII from deactivating factors such as thrombin, and helps the FVIII to have a stable structure. However, when FVIII and full-length vWF are expressed together outside cells, they co-localize in the cells, thereby resulting in inhibited secretion of FVIII. Accordingly, a mutant vWF is desired which maximizes the function of FVIII and comprises only the portion not inhibiting FVIII secretion.

Different mammalian expression vectors may be used to deliver FVIII and vWF. But, lentiviral-based vectors are preferred for the cells to which the delivery of gene is not easy, for example, stem cells, hematopoietic progenitor cells, and the like. However, lentiviral-based vectors have a size limit for the expressed genes. BDD.FVIII has a size of 4.4 Kb, whereas vWF has a size of 5.6 Kb up to the A2 domain. Accordingly, the gene can be expressed with no significant loss in the viral titer. Further, in order to increase viral titer, it is possible to pseudotype the envelope protein of lentivirus with VSV-G and then concentrate the virus.

The animal cell expression vector of the present invention may include a promoter derived from eukaryotic or prokaryotic cells that can induce transcription of foreign genes in animal cells. The promoter may include control elements for enhancement or repression of transcription. Suitable promoters may include cytomegalovirus promoter (pCMV), Rous sarcoma virus long terminal repeat promoter (pRSV), and SP6, T3 or T7 promoters. Enhancer sequences upstream from the promoter or terminator sequences downstream of the coding region may be optionally included in the vector of the present invention in order to facilitate expression. The vector of the present invention may further contain additional nucleotide sequences such as a polyadenylation sequence, a localization sequence or a signal sequence, sufficient to permit a cell to efficiently and effectively process the protein expressed by the nucleic acid of the vector. Examples of preferred polyadenylation sequences are SV40 early region polyadenylation site [C. V. Hall et al., *J. Molec. App. Genet.* 2, 101(1983)] and SV40 late region polyadenylation site [S. Carswell and J. C. Alwine, *Mol. Cell Biol.* 9, 4248(1989)]. Such additional sequences are inserted into the vector such that they are operably linked with the promoter sequence, if transcription is desired, or additionally with the initiation and processing sequences, if translation and processing are desired. Alternatively, the inserted sequences may be placed at any position in the vector. The term "operably linked" is used to describe a linkage between a gene sequence and a promoter or other regulatory or processing sequence such that the transcription of the a gene sequence is directed by an operably linked promoter sequence, the translation of the gene sequence is directed by an operably linked translational regulatory sequence, and the post-translational processing of the gene sequence is directed by an operably linked processing sequence.

Standard techniques for the construction of the vector of the present invention are well-known to those skilled in the art and can be found in such references as Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd Ed. (Cold Spring Harbor, N.Y., 1989). A variety of strategies are available for ligating DNA fragments, the choice of which depends on the nature of the terminal of the DNA fragments and can be readily made by those skilled in the art.

Examples of the lentivirus that can be used in the present invention may include HIV-1 and HIV-2, SIV, FIV, BLV, EIAV, CEV and visna viruses. Particularly, HIV and SIV are desired for gene therapy. HIV-1 (human immunodeficiency virus type 1) is a lentivirus belonging to the retrovirus family. Like other members of the family, HIV can infect non-dividing cells. This makes lentiviruses a good candidate vector for gene therapy.

HIV-1-based vectors are the most frequently used as gene delivery vehicles due to their ability to infect dividing and non-dividing cells with their cytoplasmic and nuclear entry proteins (Kohn, 2001, *J. Intern. Med.* 249, 379-390). This ability is frequently attributed to various features of the vectors, including the nuclear localization signals in multiple virion proteins and the central polypurine tract that generates a triple stranded DNA flap' in the reverse-transcribed genome. As a consequence of these features, bioengineered HIV-1 is capable of infecting hematopoietic progenitor cells very efficiently at fairly low MOIs (Park and Choi, 2004, *Mol. Cells* 17, 297-303). The primary concern with regard to the use of lentiviral vectors as tools for gene therapy is that the transfer vector is derived from HIV-1. However, all of the viral components required for viral replication were deleted in the viral vectors utilized in the present study and the transfer vector ultimately harbored less than 5% of the HIV-1 genome. Another barrier encountered when using lentiviral vectors is restriction on the size of the transferred gene. vWF comprises 52 exons with a cDNA size of approximately 9 Kb, which exceeds the size limit of the majority of lentiviral vectors. In this report we successfully forced vWF cDNA into a lentiviral vector (FIGS. **1** and **2**). In the preparation and production of the lentivirus, we substituted the env of HIV-1 with the VSV-G protein. VSV-G mediates viral entry into cells via membrane fusion rather than a specific cell surface receptor protein, resulting in a significant broadening of the host range (Hofmann et al., 1999, *J. Virol.* 12, 10010-10018). More importantly, it confers structural stability during ultracentrifugation, enabling concentration of the virus to high titers with no significant loss of infectivity (Burns et al., 1993; Hofmann et al., 1999). By exploiting these features of VSV-G, we successfully produced and concentrated vEx52, resulting in six fold higher transduction efficiency with only 1/100th of the volume of lentiviral supernatant (FIG. **3**). These results were FACS (Fluorescence-activated cell sorting) analyzed and clearly observed under fluorescence light: significantly greater quantities of eGFP were observed in the cells transduced with the concentrated vEx52 than with the non-concentrated vEx52 (FIG. **4**). Recent work by De Meyer et al. involved incorporation of a long vWF cDNA into a lentiviral vector and transduction of blood-outgrowth endothelial cells (BOECs) from von Willebrand disease type 3 dogs to develop gene therapy with type 3 VWD (De Meyer et al., 2006, *Blood* 107, 4728-4736). However, concentrating low titers of virus may not prove to be ideal for actual application in the treatment of hemophilia A as it requires additional time-consuming and laborious procedures. Therefore, we attempted to reduce the size of the vWF cDNA insert in the lentiviral vector. We deleted domains of vWF leaving only minimal regions for interactions between vWF and FVIII. The mature vWF consists of the D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2 domains. FVIII binds to the D'-D3 domain, and the AI domain binds to platelet glycoprotein Ib, heparin, and collagen. This facilitates the aggregation of platelets and also aids in adhesion to sites of vascular injury. The vWF gene is located on chromosome 12 and comprises 52 exons with 178,000 bases. We deleted exons 24-46 to create pRex23 and pvEx23, thus retaining only the region that binds to FVIII. FVIII binds to vWF within the 272 amino acid residues located at its amino terminus (Sadler, 1998).

We also constructed pRex28 and pvEx28, in which exons 29-46 are deleted, thereby leaving the platelet binding sites in addition to the FVIII binding region. The platelet binding site on vWF is located within the A1 domain (Sadler, 1998). When pvEx23, pvEx28 and pvEx52 were packaged into lentiviruses, virus production from pvEx23 and pvEx28 was significantly greater than from pvEx52. Generally, the viral titer of non-concentrated vEx52 was 2×10^4 to 4×10^4 particles/ml (FIG. **3**), whereas the titers of vEx23 and vEx28 were between 1×10^5 and 3×10^5 particles/ml (FIG. **5**). The transduction efficiencies of the three viruses can be compared from the histograms in FIGS. **3** and **5**. When 500 μl of vEx23, vEx28, and vEx52 was used to transduce Jurkat cells, 35.02%, 26.30% and 4.64% of the cells, respectively, were positive for eGFP. Therefore, we were able to improve viral titers and transduction efficiencies by deleting the domains within vWF that are less important for the interaction with FVIII, thus reducing the packaging size. When pRex23, pRex28 and pRex52 were transfected into 293T cells and functional FVIII was measured in the supernatants, pRex23 and pRex28 had lower FVIII activity than observed with the full-length vWF, pRex52. However, using the viral system, the supernatants from the cells transduced with vEx28 had higher secreted BDD.FVIII activity than those from vEx52 (FIG. **6**). This may be because the large size of the full-length vWF limits the efficiency its packaging and expression. While we cannot decide whether the expression of FVIII was altered by vWF, vEx28 increased the secreted level of expressed FVIII in the supernatants, and this effect is most likely attributable to protection of the conformation of BDD.FVIII. This is consistent with the observation that more FVIII activity was detected in cells when vWF was present (Kaufman et al., 1997, *Blood* 8, S3-14). Another indication that vWF stabilizes FVIII is the fact that the FVIII was degraded rapidly in the absence of vWF (Over et al., 1978, *J. Clin. Invest.* 62, 223234), whereas it was cleared more slowly in the presence of vWF (Tuddenham et al., 1982, *Br. J. Haematol.* 52, 259-267). With greater insight into the nature of vWF and FVIII, the two proteins may be engineered to provide a powerful genetic tool for correcting FVIII-deficient cells.

Pharmaceutical formulations of the present invention include those suitable for parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous and intra-arterial), oral or inhalation administration. Alternatively, pharmaceutical formulations of the present invention may be suitable for administration to the mucous membranes of a subject (e.g., intranasal administration). The formulations may be conventionally prepared in unit dosage form and may be prepared by any of the methods well known in the art.

The dosage of the pharmaceutical formulations of the present invention may vary depending on the formulation type, administration method, age, body weight and sex of the subject, severity of disease, diet, administration time, administration route, rate of excretion, response sensitivity, or the like. A skilled physician will readily determine a dosage effective for the desired treatment. In general, the pharmaceutical composition of the present invention is administered with a unit dosage of 10^3-10^7 viral particles or 0.001-100 mg/kg of protein.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other features and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in:

FIG. **1** schematically represents the packaging constructs comprising a mutant von Willebrand Factor (vWF) gene according to an embodiment of the present invention;

FIG. **2**A-**2**B illustrate integration (gDNA, FIG. **2**A) and transcription (cDNA, FIG. **2**B) of vWF from transduced COS-1 cells gene according to an embodiment of the present invention;

FIG. **3** concentration of vWF-expressing HIV-1 according to an embodiment of the present invention;

FIG. **4**A-**4**E illustrate transduction of pseudotyped HIV-1 expressing vWF according to an embodiment of the present invention. Transfection of the vectors resulted in lentivirus production, as indirectly confirmed by expression of eGFP (FIG. **4**A). The transduction and expression of both the non-concentrated and the concentrated vEx52 were also visualized with fluorescence light from the eGFP expression (FIG. **4**B-D). In order to verify vWF expression from vEx52, COS-1 cells were transduced with vEx52 at a MOI of 0.5 and labeled with human vWF antibody, followed by TRITC staining (FIG. **4**E).;

FIG. **5**A-B schematically represent the deletion constructs of vWF according to an embodiment of the present invention. pRex23 and pRex28 were generated from pREP7-vWF (generously provided by Dr. Subrata Banerjee) by deleting exons 24-46 and 29-46, respectively, and pvEx23 and pvEx28 were generated in the same way from pvEx52 (FIG. **5**A). The sequences were deleted by PCR using the forward primer 5'-CGTGATGAGACGCTCCAG-3' (SEQ ID NO.: 17), and the reverse primer of Ex23PR 5'-TTTTCTGGTGTCAGCACACTG-3' (SEQ ID NO.: 18) for pRex23 and pvEx23, and Ex28PR 5'-CAGGTGCAGGGGAGAGG-3' (SEQ ID NO.: 19) for pRex28 and pvEx28. pvEx23 and pvEx28 were then used to generate VSV-G pseudotyped HIV-1 with packaging vectors, and titrated in Jurkat cells. 35.02% and 26.30% of the cells proved to be positive for eGFP when 500 μl of the viral supernatants of vEx23 and vEx28, respectively, were employed for transduction (FIG. **5**B).;

FIG. **6**A-C illustrate detection of the activity of secreted functional coagulation Factor VIII (FVIII) according to an embodiment of the present invention. Levels of FVIII activity of 28.89±18.86, 107.22±30.64, and 199.44±58.93 were obtained from transfection with pRex23, pRex28, and pRex52, respectively (FIG. **6**A). FVIII activity in the supernatants of the transduced cells was 28.33±5.50 for the vEx52-transduced cells, and 33.89±3.93 and 53.33±9.43, respectively for the vEx23- and vEx28-transduced cells (FIG. **6**B). K562 cells were co-transduced with vBDD.FVIII, the BDD-FVIII expressing HIV-1, along with vEx23, vEx28, or vEx52 at a MO1 of 1.5. RT-PCR with RNA from the transduced cells confirmed expression of the transduced FVIII (FIG. **6**C);

FIG. **7** illustrates expression of eGFP tested to confirm the expression of FVIII according to an embodiment of the present invention;

FIG. **8** illustrates activation of FVIII by different mutant vWFs determined by chromogenic assay according to an embodiment of the present invention (FIG. **8**A represents activities of FVIII in normal state (without damage) and FIG. **8**B represents activities of FVIII treated with PMA (phorbol ester) in damaged state);

FIG. **9** illustrates a process of manufacturing a lentiviral vector comprising a B-domain-deleted FVIII gene according to an embodiment of the present invention;

FIG. **10** illustrates a process of manufacturing a lentiviral vector comprising a vWF gene according to an embodiment of the present invention;

FIG. **11** illustrates a process of manufacturing vectors comprising pRex23, pRex28 and pRex32 comprising variants of vWF according to an embodiment of the present invention;

FIG. **12** illustrates a process of manufacturing lentiviral vectors pvEx23, pvEx28 and pvEx32 comprising variants of vWF according to an embodiment of the present invention; and

FIG. **13** illustrates a process of manufacturing a pvBDD.FVIII.vWEx32 lentiviral vector comprising BDD.FVIII and vWF variant genes.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in more detail through examples. However, the following examples are only for the illustration of the present invention and the scope of the present invention is not to be construed as limited by them.

Example 1

Construction of Vectors

Plasmid pRF8 was obtained by cloning full length FVIII cDNA (ATCC® Accession No. 40086) into Not1 site of a modified pREP7 vector (Invitrogen™, USA) using a linker. Then, in order to delete most of B-domain, the upstream 5' region of B-domain was amplified by PCR using primers, 5'-GAACCGAAGCTGGTACCT-3' (SEQ ID NO.: 9) and 5'-GACAGGAGGGGCATTAAATTGCTTTTGCCT-3' (SEQ ID NO.: 10), and the downstream 3' region was amplifiedusing primers, 5'-TTTAATGCCCCACCAGTCTTGAAACGCCAT-3' (SEQ ID NO.: 11) and 5'-ATGCTCGCCAATAAGGCATTCCA-3' (SEQ ID NO.: 12). Then, the amplified products were denatured with heat and renatured to obtain the product. The resulting product was cleaved by Kpn1 and Bgl1 and sub-cloned into Kpn1-Bgll of pRF8 plasmid to produce pREP7-BDD.FVIII in which B-domain-deleted (BDD) FVIII cDNA was inserted under the RSV 3' LTR control of pREP7 (Invitrogen™, USA) (see The Journal of Gene Medicine, Volume 6, Issue 7, Pages 760-768). We used the pREP7-BDD.FVII from Subrata Banerjee, an author of the thesis. Besides, pRex52 plasmid was obtained by cloning full length vWF cDNA (ATCC® #59126) into Not1 site of a modified pREP7 vector (Invitrogen™, USA) using a linker as described above.

pHIvec2.GFP obtained from Joseph Sodroski, an author of Journal of Virology, December 1999, p. 10020-10028, was used as a Lentivirus backbone (transfer vector) in the invention. The pHIvec2.GFP was prepared by deleting env and vpu sequences from v653 rtatpC virus, maintaining Rev-responsive element, and inserting eGFP gene (Clontech, USA) after IRES. The vector map of pHIvec2.GFP is the same with a vWF gene-deleted form of FIG. **1**.

To manufacture a Lentivirus vector comprising B-domain-deleted FVIII gene, cDNA of BDD.FVIII (B-Domain-Deleted Coagulation Factor VIII) was obtain from the pREP7-BDD.FVIII using Not1. The cDNA was inserted into the lentivirus backbone using the same enzyme to produce pvBDD.FVIII. This process of manufacturing is represented in FIG. **9**. In detail, pRep7-BDD.FVIII and lentivirus backbone were digested by Not1 and the BDD.FVIII fragments from pREP7-BDD.FVIII were ligated in Not1 site of lentivirus backbone.

To manufacture Lentivirus vector comprising vWF (von Willebrand Factor) gene, the pRex52 and lentivirus backbone were digested by Not1, vWF fragments were ligated in Not1 site of lentivirus backbone, and pvEx52 was manufactured. This process of manufacturing was represented in FIG. **10**. A particular map of pvEx52 as a result of FIG. **10** is shown in FIG. **1**. vWF gene is located between long terminal repeats (LTRs) and fused into IRES-eGFP of a viral vector. vWF variants were manufactured from the pRex52 using PCR (polymerase chain reaction). The PCR was performed using dNTP 25 mM, phosphorylated primers 10 μg, 2 mM Mg^{2+}, DNA template, and pfuUltraTMII Fusion™ HS DNA Polymerase (Agilent Technologies, USA). The PCR had 50 μg of Total volume and was performed under following conditions: 5 min at 95° C.; 18 cycles of 30 sec at 95° C., 30 sec at 52° C. and 30 sec at 72° C. per 1 Kb; and 10 min at 72° C. For manufacturing the vWF variants, a forward primer sequence was 5'-CGT GATGAGACGCTCCAG-3' (SEQ ID NO.: 13), and reverse primer sequences were 5'-TTTTCTGGTGTCAGCACACTG-3' (SEQ ID NO.: 14; pRex23), 5'-AGGTGCAGGGGAGAGGGT-3' (SEQ ID NO.: 15; pRex28) and 5'-AGAGCACAGTTTGTGGAG-3' (SEQ ID NO.: 16; pRex32), respectively. After PCR, amplified products were isolated by using PCR removal kit (Qiagen, USA) and ligated with ligase (Takara, JAPAN) at 15° C. for approximately 24 hour to manufacture pRex23, pRex28 and pRex32. This process of manufacturing is shown in FIG. **11**. The ligated mixture was transformed into TOP10.

To manufacture Lentivirus vector comprising vWF variants gene, the pRex23, pRex28 or pRex32 and lentivirus backbone were digested by Not1, the vWf fragments from the pRex23, pRex28 or pRex32 were ligated into Not1 site of lentivirus backbone, and pvEx23, pvEx28 and pvEx32 were manufactured. This process of manufacturing is shown in FIG. **12**.

pvBDD.FVIII has a form that IRES-eGFP is located after the BDD.FVIII. Only the eGFP was deleted from the pvBDD.FVIII by PCR and thereinto vWF variant was inserted to produce pvBDD.FVIII.vWEx32. This process of manufacturing is shown FIG. **13**.

The TOP10 was used as a host in the transformation. To co-express BDD.FVIII and vWF21, IRES (internal ribosomal entry site) sequence was inserted after BDD.FVIII and thereafter vWF32 was inserted. Therefore, co-expression of two proteins under one promoter is possible, and the vWF32 expressed after BDD.FVIII plays a role in helping an activity and function of BDD.FVIII.

Example 2

Production of Virus

Vesicular stomatitis G protein (VSV-G) pseudotyped HIV-1 was produced by cotransfecting 293T cells with gag-pol, tat, rev, VSV-G and transfer vector using quinquepartite plasmid transient transfection method (Park and Choi, 2004 *Mol. Cells* 17, 297-303). 293T cells were subcultured at a density of 4.5×10^6 cells on 100 mm plates 24 hours prior to transfection. The supernatant was replaced with culture medium comprising 10% FCS and 25 mM HEPES 4 hours prior to transfection. For transfection, packaging plasmid with Gag and Pol 10 μg, VSV-G plasmid 2 μg, Tat plasmid 1 μg, Rev plasmid 1 μg and transfer vector 10 μg were used. These DNAs were added in 62 μl of 2.5 M $CaCl_2$, the volume was set to 500 μl with water, and vortexed. This mixture was added with 500 11R of 2×HBS (281 mM NaCl, 100 mM HEPES, 1.5 mM Na_2HPO_4 pH 7.12), left for 30 min at room temperature, and then spread on 293T cells. 16 hours after transfection, the supernatant was replaced with RPMI of 10 mM HEPES buffer. After 48 hours, viruses produced and flowed to supernatant were harvested by using 0.45 μm filter.

Example 3

Titration of Virus

3×10^5 cells of NIH3T3 cells were placed on 60 cm^2 dishes, and after 20 hours serial dilutions of viral stocks were added in the cells. Total volume was set to 2 ml and 2 μg/ml of polybrene (Sigma-Aldrich, USA) was added. After 6 hours, the virus was removed, the cells were washed with DMEM comprising 2% FCS to remove the virus completely, and the cells were put into an incubator. After 2 days, the cells were separated with 0.25% trypsin, washed with 1×PBS, and fixed with 3.7% formaldehyde. Percent of $eGFP^+$ radiating in the infected cells was determined using FACScan™ (Becton Dickinson, USA Immunocytometry System) and CellQuest program (Becton Dickinson, USA), and then the titer of virus was calculated using the following formula: (2× a number of cells×Percent of $eGFP^+$ cells)+quantity of virus.

Example 4

Concentration of Virus

The filtered virus was transferred to polyallomer tubes and ultracentrifuged at 50,000×g in SW28 rotor for 1.5 hour at 4° C. The pellet was resuspended in a small volume of medium. Then, the tube was covered with Parafilm®, and left to stand at 4° C. for 24 hours. For extended storage, the viral stocks were stored at −80° C. FIG. **3** represents concentration of vWF-expressing HIV-1 according to an embodiment of the prevent invention. 500 μl of non-concentrated (middle) and 5 μl of concentrated (right) of vWF-expressing lentivirus supernatants were used to transduce Jurkat cells. The fraction of eGFP+ cells among the transduced cells was determined by flow cytometry.

Example 5

Transduction of Cells

The cells were counted by hemocytometer and plated in 24-well or 6-well plates at the desired cell number. Viral supernatants were added to the cells at the desired multiplicity of infection (MOI). At this time, the total volume was adjusted to the desired volume with the culture medium, and polybrene was added at a concentration of 2 μg/ml. The infection was performed in the presence of 5% CO^2 for 6 hours at 37° C. After infection, the cells were washed with the medium. FIG. **5** is a schematic showing deleted constructs of vWF according to an embodiment of the prevent invention. (A) pRex23 and pvEx23 were constructed by deleting exons 24-46, and pRex28 and pvEx28 were generated by deleting exams 29-46, from pRex52 and the lentiviral vector, respectively. (B) vEx23 and vEx28 were generated from pvEx23 and pvEx28, respectively, and 500 μl of viral supernatants were used to transduce Jurkat cells. The percentages of cells transduced were analyzed by FACS (Fluorescence-activated cell sorting).

Example 6

Isolation of DNA and RNA

Genomic DNA was prepared with 500 μl of lysis buffer (0.1 M Tris HCl, pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl and 100 μg/ml protease K). It was precipitated with isopropanol and washed with 75% ethanol. RNA was prepared with Trizol® reagent (Invitrogen™, USA) and cDNA was synthesized using ImPromII™ (Promega, USA). PCR was carried out with Pfu (SolGent, Republic of Korea) in a total volume of 50 μl containing 1× reaction buffer, 25 mM each dNTP, 10 μM each primers, 2 mM Mg^{2+}, and DNA template. FIG. **2** represents integration and transcription of vWF from transduced COS-1 cells according to an embodiment of the prevent invention. The COS-1 cells were transduced with lentivirus expressing vWF. (A) Integration of the transduced vWF gene was detected in the genomic DNA of transduced COS-1 cells by PCR for 421-bp of vWF and 227-bp of LTR. (B) cDNA was prepared from the transduced cells and amplified with primers specific for 421-, 227- and 187-bp of vWF, LTR, and GAPDH, respectively. vEx52: transduced with vEx52, eGFP: transduced with eGFP-expressing lentivirus.

Example 7

Plasmid Transfection

DNAs (pRex23, pRex28 and pRex32) for transfection were added into 50 μl of 150 mM NaCl on 12-well plate, vortexed and spun down. This mixture was added with PEI in 3 times volume of DNA, vortexed and spin down again. This mixture was left for 10 min at room temperature, and was dropped on the cells carefully.

Example 8

Immunocytochemistry

Transduced cells were grown on glycogen-coated coverslips in 6-well tissue culture plates. The cells were fixed in cooled 100% methanol, washed with TBS [50 mM Tris-HCl (pH 7.4), 150 mM NaCl], quenched in fresh 0.1% sodium borohydride in TBS for 5 min and washed three times with TBS for 5 min. The cells were blocked with blocking buffer (10% horse serum, 1% bovine serum albumin, 0.02% NaN_3 in 1×PBS) for 60 min and washed for 5 min with TBS. Primary vWF antibody (Abcam®, USA) was diluted in 1% BSA in TBS and incubated overnight with the cells at 4° C. After washing the cells three times for 5 min with TBS, they were labeled with secondary goat-antimouse IgG TRITC antibody (Santa Cruz Biotechnology, USA) in 1% BSA in TBS for 30 min at room temperature covered with aluminum foil. They were then washed 3 times with TBS for 5 min and mounted on slides using a ProLong® Antifade Kit (Cell Signaling, USA).

FIG. **4** represents transduction by pseudotyped HIV-1 expressing vWF according to an embodiment of the prevent invention. (A) 293T cells were cotransfected with plasmids harboring viral components for virus production including a transfer vector that harbored vWF-IRES-eGFP. eGFP from the transfer vector was visualized under a fluorescence microscope at 10× magnifications. (B) Jurkat cells were visualized under a microscope with bright and fluorescence light. (C) Jurkat cells were transduced with 500 μl of non-concentrated vWF-expressing pseudotyped HIV-1 and visualized under a microscope. (D) Jurkat cells were transduced with 5 μl of 160-fold concentrated vWF-expressing pseudotyped HIV-1 and visualized under the white and fluorescence light. (E) COS-1 cells were transduced with vWF-pseudotyped HIV-1 at a MOI of 0.5. eGFP was visualized under a fluorescence microscope (left). vWF was visualized by staining vWF with antibody and TRITC (middle). The detected vWF was not from the COS-1 cells but from the transferred gene (right).

BDD.FVIII deleting B domain from sequence of FVIII was prepared and inserted into lentivirus vector. For expression of lentivirus, the BDD.FVIII was expressed together with gag, pol, VSV-G, tat, and rev in 293T cells to produce lentivirus expressing BDD.FVIII. The transfected cells may express BDD.FVIII. Conclusively, the expression was confirmed by measuring activity of FVIII. Production and infection of virus were confirmed indirectly by expression of eGFP in the vector. FIG. **7** is a photograph representing result of eGFP expression in order to confirm expression of FVIII according to an embodiment of the prevent invention (correspond to FIG. **4** (*a*)). Production and infection of virus were confirmed indirectly by expression of eGFP.

Example 9

Measurement of Factor VIII Activity

Activated FVIII activity (FVIII:C) was measured by Coatest® VIII:C/4 kit (DiaPharm, Italy). One volume of phospholipids and 100 mg/l ciprofloxacin was mixed with 5 volumes of Factor IXa and Factor X. The mixture 50 μl was placed in 96-well microtiter plates, added with 25 μl of cell culture supernatant, incubated for 5 min at 37° C. And then, the mixture was added with 25 μl of 0.025 mol/L CaCl2, incubated for 5 min at 37° C., added with 50 μl of S-2765 and 1-2581, and incubated for 10 min at 37° C. The incubation was stopped by 20% acetic acid, and the activity of FVIII was measured at 405 nm. A standard curve was made with each experiment using known amounts of recombinant human FVIII.

FIG. **6** represents detection of the activity of secreted functional FVIII. (A) 293T cells were co-transfected with pREP7-BDD.FVIII and pRex23, pRex28, or pRex52. The supernatants of the transfected cells were collected and quantitated for FVIII activity. (B) K562 cells were co-transduced with HIV-1-BDD.FVIII and vEx23, vEx28 or vEx52 at a MOI of

1.5. The supernatants of the transduced cells were collected and screened for FVIII activity. The data are expressed as the means±S.E. of at least three independent experiments. (C) RT-PCR was performed with RNAs from the transduced cells. B-domain-deleted FVIII yields a product of 1.1 Kb. vEx23: transduced with vEx23, vEx28: transduced with vEx28, vEx52: transduced with vEx52.

Example 10

Measurement of Factor VIII Activity after PMA Treatment

pRex23, pRex28 and pRex32 are named for Von Willebrand Factors deleting C-terminal domains from exon 23, exon 28 and exon 32, respectively. pRex52 is named for full length of vWF. They were co-expressed with pREP7-BF carrying a von Willebrand factor vector and the activity of secreted FVIII was determined. As a result, pREP7-BF and pRex32 had the greatest activity of secreted FVIII (FIG. **8**). FIG. **8** represents the results of measuring the activity of FVIII for each vWF variant by Chromatography Assay. HeLa cells were transfected with pREP7; pREP7-BF and pRex23; pREP7-BF and pRex28; pREP7-BF and pRex32; and pREP7-BF and pRex52, and then functional activities of FVIII secreted out in supernatant were measured. FIG. **8**A represents the results of measuring the activities of FVIII in normal state (without damage). The activities of all FVIIIs were increased as compared with a basal level, and the 32 (pREP7-BF and pRex32) was the greatest. FIG. **8**B represents the activities of FVIII treated with PMA (phorbol ester) in damaged state. The secretion of all FVIIIs was induced at least 3 times and marked a remarkable difference as compared with basal level. The result means that FVIII secretion is induced greatly in damaged state, but not in normal state, which give a very useful advantage in real clinics of hemophilia.

Result 1: Lentivirus Production

8.8 Kb of von Willebrand Factor cDNA was cloned into the HIV-1-based lentivirus between the two long terminal repeats to create pvEx52 (LTRs) (FIG. **1**). The vWF cDNA was excised from vW-8 (ATCC® #59126) using EcoR1 and Sac1 and cloned into the lentiviral vector. The vWF gene was fused with IRES-eGFP, thus permitting the use of enhanced green fluorescence protein (eGFP) as an indirect indicator of virus production after transfection into 293T cells together with the other viral genes required for packaging viral particles (Parolin et al., 1996; Yee et al., 1994). The packaging vector harbored gag and pol under the control of the CMV promoter, and the tat and rev of HIV-1 were expressed separately under the control of the CMV promoter. We used the vesicular stomatitis virus G protein (VSV-G) instead of HIV-1 env (Hofmann et al., 1999).

Result 2: Transduction of VSV-G Pseudotyped HIV-1

COS-1 cells were transduced with vEx52, the lentivirus carrying the complete vWF, or HIV-1-eGFP, the eGFP-expressing lentivirus. Genomic DNA was isolated from the transduced COS-1 cells, and PCR was conducted using primers specific for the human vWF gene and HIV-1 LTR. vWF could be amplified only from the vEx52-transduced cells, whereas LTR could be amplified from both vEx52 and HIV-1-eGFP-transduced cells (FIG. **2**A). In addition, RNA was prepared from the transduced cells, with which cDNA was synthesized and PCR for vWF and LTR was conducted (FIG. **2**B). The result of RT-PCR accorded with the amplifications from the PCR of the genomic DNA. vWF was amplified only from the vEx52-transduced cells, and LTR was detected from both vEx52 and HIV-1-eGFP-transduced cells.

Result 3: Expression of vWF from Transduced Lymphoblast Cells and Concentration of vEx52

Jurkat cells were plated at 2×10^5 and transduced with 500 μl of non-concentrated vEx52 virus. FACS analysis showed that 5.55% of the cells were positive for GFP expression (FIG. **3**). After the virus suspension was concentrated by a factor of 160 by ultracentrifugation at 50,000×g, 5 μl of concentrated virus was employed to transduce an equal number of Jurkat cells, resulting in a yield of 29.51% eGFP+ (FIG. **3**). By concentrating the vEx52 virus, the titer increased from 2.8×10^4 particles/ml to 2.3×10^7 particles/ml. Transfection of the vectors resulted in lentivirus production, as indirectly confirmed by expression of eGFP (FIG. **4**A). The transduction and expression of both the non-concentrated and the concentrated vEx52 were also visualized with fluorescence light from the eGFP expression (FIG. **4**B-D). In order to verify vWF expression from vEx52, COS-1 cells were transduced with vEx52 at a MOI of 0.5 and labeled with human vWF antibody, followed by TRITC staining (FIG. **4**E). In addition, in order to determine whether the transduced constructs were maintained for an extended period of time, 1×10^5 Jurkat cells were transduced at a MOI of 0.5. After 4 days, 38.27% of the cells were shown by FACS analysis to be positive for eGFP. When analyzed on days 9, 15, 35, 50 and 90 post-transduction, 33.01%, 11.99%, 11.32%, 6.13%, and 5.56%, respectively of the cells were eGFP+(data not shown).

Result 4: Construction of Domain-Deleted vWF

pRex23 and pRex28 were generated from pREP7-vWF (generously provided by Dr. Subrata Banerjee) by deleting exons 24-46 and 29-46, respectively, and pvEx23 and pvEx28 were generated in the same way from pvEx52 (FIG. **5**A). The sequences were deleted by PCR using the forward primer 5'-CGTGATGAGACGCTCCAG-3' (SEQ ID NO.: 17), and the reverse primer of Ex23PR 5'-TTTTCTGGTGTCAGCACACTG-3' (SEQ ID NO.: 18) for pRex23 and pvEx23, and Ex28PR 5'-CAGGTGCAGGGGAGAGG-3' (SEQ ID NO.: 19) for pRex28 and pvEx28. pvEx23 and pvEx28 were then used to generate VSV-G pseudotyped HIV-1 with packaging vectors, and titrated in Jurkat cells. 35.02% and 26.30% of the cells proved to be positive for eGFP when 500 μl of the viral supernatants of vEx23 and vEx28, respectively, were employed for transduction (FIG. **5**B).

Result 5: Functional Activity of the Secreted FVIII

In order to examine the effects of domain-deleted vWF on the secretion of FVIII, 293T cells were transfected with pREP7-BDD.FVIII along with one pRex23, pRex28, or pRex52. After 48 h of transfection, the supernatants were collected and screened for functional FVIII activity in chromogenic assays. Levels of FVIII activity of 28.89±18.86, 107.22±30.64, and 199.44±58.93 were obtained from transfection with pRex23, pRex28, and pRex52, respectively (FIG. **6**). Therefore, the functional activity of the secreted FVIII declined as more of vWF was deleted. Next, we assessed the effects of domain-deleted vWF when it was transduced as a component of vWF lentivirus. K562 cells were co-transduced with vBDD.FVIII, the BDD-FVIII expressing HIV-1, along with vEx23, vEx28, or vEx52 at a MO1 of 1.5. RT-PCR with RNA from the transduced cells confirmed expression of the transduced FVIII (FIG. **6**). FVIII activity in the supernatants of the transduced cells was 28.33±5.50 for the vEx52-transduced cells, and 33.89±3.93 and 53.33±9.43, respectively for the vEx23- and vEx28-transduced cells (FIG. **6**). These data suggest that the deleted form of vWF, vEx28, is the most efficient at promoting secretion of FVIII via interaction of its minimal essential domains with FVIII.

As described, in accordance with the present invention, coagulation Factor VIII (FVIII) can be effectively expressed in a viral vector and the FVIII activity can be significantly enhanced using mutant von Willebrand Factor (vWF) with a reduced size. Further, the viral vector of the present invention may be effectively used to treat hemophilia through gene therapy. The coexpression of FVIII and vWF may be very useful in clinical applications such as gene therapy for hemophilia A treatment.

While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by appended claims.

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<210> SEQ ID NO 4
<211> LENGTH: 1833
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly
35 40 45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly
50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys
65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu
85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro
100 105 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys
115 120 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly
130 135 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly
145 150 155 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln
165 170 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala
180 185 190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser
195 200 205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln
210 215 220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu
225 230 235 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu
245 250 255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala
260 265 270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His
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Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys
290 295 300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met
305 310 315 320

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Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His
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Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn
        355                 360                 365

Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys
    370                 375                 380

Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp
385                 390                 395                 400

Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg
                405                 410                 415

Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys
            420                 425                 430

Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu
        435                 440                 445

Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val
    450                 455                 460

Ala Met Asp Gly Gln Asp Val Gln Leu Pro Leu Leu Lys Gly Asp Leu
465                 470                 475                 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu
                485                 490                 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu
            500                 505                 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn
        515                 520                 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro
    530                 535                 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln
545                 550                 555                 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met
                565                 570                 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe
            580                 585                 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys
        595                 600                 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly
    610                 615                 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val
625                 630                 635                 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln
                645                 650                 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu
            660                 665                 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe
        675                 680                 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys
    690                 695                 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp
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Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met
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His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val
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Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg
        755                 760                 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu
    770                 775                 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met
785                 790                 795                 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg
                805                 810                 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln
            820                 825                 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr
        835                 840                 845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr His Val Cys Asp Ala
    850                 855                 860

Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly Leu
865                 870                 875                 880

Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp Tyr
                885                 890                 895

Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys Gly
            900                 905                 910

Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu Val
        915                 920                 925

Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys Arg
    930                 935                 940

Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg Tyr
945                 950                 955                 960

Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg His
                965                 970                 975

Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val Cys
            980                 985                 990

Gly Leu Cys Gly Asn Phe Asp Gly  Ile Gln Asn Asn Asp  Leu Thr Ser
        995                 1000                 1005

Ser Asn  Leu Gln Val Glu Glu  Asp Pro Val Asp Phe  Gly Asn Ser
    1010                 1015                 1020

Trp Lys  Val Ser Ser Gln Cys  Ala Asp Thr Arg Lys  Val Pro Leu
    1025                 1030                 1035

Asp Ser  Ser Pro Ala Thr Cys  His Asn Asn Ile Met  Lys Gln Thr
    1040                 1045                 1050

Met Val  Asp Ser Ser Cys Arg  Ile Leu Thr Ser Asp  Val Phe Gln
    1055                 1060                 1065

Asp Cys  Asn Lys Leu Val Asp  Pro Glu Pro Tyr Leu  Asp Val Cys
    1070                 1075                 1080

Ile Tyr  Asp Thr Cys Ser Cys  Glu Ser Ile Gly Asp  Cys Ala Cys
    1085                 1090                 1095

Phe Cys  Asp Thr Ile Ala Ala  Tyr Ala His Val Cys  Ala Gln His
    1100                 1105                 1110

Gly Lys  Val Val Thr Trp Arg  Thr Ala Thr Leu Cys  Pro Gln Ser
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Cys Glu  Glu Arg Asn Leu Arg  Glu Asn Gly Tyr Glu  Cys Glu Trp
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Arg Tyr  Asn Ser Cys Ala Pro  Ala Cys Gln Val Thr  Cys Gln His
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Pro Glu  Pro Leu Ala Cys Pro  Val Gln Cys Val Glu  Gly Cys His
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Ala His  Cys Pro Pro Gly Lys  Ile Leu Asp Glu Leu  Leu Gln Thr
    1175                 1180                 1185

Cys Val  Asp Pro Glu Asp Cys  Pro Val Cys Glu Val  Ala Gly Arg
    1190                 1195                 1200

Arg Phe  Ala Ser Gly Lys Lys  Val Thr Leu Asn Pro  Ser Asp Pro
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Glu His  Cys Gln Ile Cys His  Cys Asp Val Val Asn  Leu Thr Cys
    1220                 1225                 1230

Glu Ala  Cys Gln Glu Pro Gly  Gly Leu Val Val Pro  Pro Thr Asp
    1235                 1240                 1245

Ala Pro  Val Ser Pro Thr Thr  Leu Tyr Val Glu Asp  Ile Ser Glu
    1250                 1255                 1260

Pro Pro  Leu His Asp Phe Tyr  Cys Ser Arg Leu Leu  Asp Leu Val
    1265                 1270                 1275

Phe Leu  Leu Asp Gly Ser Ser  Arg Leu Ser Glu Ala  Glu Phe Glu
    1280                 1285                 1290

Val Leu  Lys Ala Phe Val Val  Asp Met Met Glu Arg  Leu Arg Ile
    1295                 1300                 1305

Ser Gln  Lys Trp Val Arg Val  Ala Val Val Glu Tyr  His Asp Gly
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Ser His  Ala Tyr Ile Gly Leu  Lys Asp Arg Lys Arg  Pro Ser Glu
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Leu Arg  Arg Ile Ala Ser Gln  Val Lys Tyr Ala Gly  Ser Gln Val
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Ala Ser  Thr Ser Glu Val Leu  Lys Tyr Thr Leu Phe  Gln Ile Phe
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Ser Lys  Ile Asp Arg Pro Glu  Ala Ser Arg Ile Thr  Leu Leu Leu
    1370                 1375                 1380

Met Ala  Ser Gln Glu Pro Gln  Arg Met Ser Arg Asn  Phe Val Arg
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Tyr Gln  Gly Leu Lys Lys Lys  Lys Val Ile Val Ile  Pro Val Gly
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Ile Gly  Pro His Ala Asn Leu  Lys Gln Ile Arg Leu  Ile Glu Lys
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Gln Ala  Pro Glu Asn Lys Ala  Phe Val Leu Ser Ser  Val Asp Glu
    1430                 1435                 1440

Leu Glu  Gln Gln Arg Asp Glu  Ile Val Ser Tyr Leu  Cys Asp Leu
    1445                 1450                 1455

Ala Pro  Glu Ala Pro Pro Pro  Thr Leu Pro Pro Asp  Met Ala Gln
    1460                 1465                 1470

Val Thr  Val Gly Pro Gly Leu  Leu Gly Val Ser Thr  Leu Gly Pro
    1475                 1480                 1485

Lys Arg  Asn Ser Met Val Leu  Asp Val Ala Phe Val  Leu Glu Gly
    1490                 1495                 1500

Ser Asp  Lys Ile Gly Glu Ala  Asp Phe Asn Arg Ser  Lys Glu Phe
    1505                 1510                 1515

Met Glu  Glu Val Ile Gln Arg  Met Asp Val Gly Gln  Asp Ser Ile
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His Val  Thr Val Leu Gln Tyr  Ser Tyr Met Val Thr  Val Glu Tyr
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Pro Phe  Ser Glu Ala Gln Ser  Lys Gly Asp Ile Leu  Gln Arg Val
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Arg Glu  Ile Arg Tyr Gln Gly  Gly Asn Arg Thr Asn  Thr Gly Leu
    1565                 1570                 1575

Ala Leu  Arg Tyr Leu Ser Asp  His Ser Phe Leu Val  Ser Gln Gly
    1580                 1585                 1590

Asp Arg  Glu Gln Ala Pro Asn  Leu Val Tyr Met Val  Thr Gly Asn
    1595                 1600                 1605

Pro Ala  Ser Asp Glu Ile Lys  Arg Leu Pro Gly Asp  Ile Gln Val
    1610                 1615                 1620

Val Pro  Ile Gly Val Gly Pro  Asn Ala Asn Val Gln  Glu Leu Glu
    1625                 1630                 1635

Arg Ile  Gly Trp Pro Asn Ala  Pro Ile Leu Ile Gln  Asp Phe Glu
    1640                 1645                 1650

Thr Leu  Pro Arg Glu Ala Pro  Asp Leu Val Leu Gln  Arg Cys Cys
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Ser Gly  Glu Gly Leu Gln Ile  Pro Thr Leu Ser Pro  Ala Pro Arg
    1670                 1675                 1680

Asp Glu  Thr Leu Gln Asp Gly  Cys Asp Thr His Phe  Cys Lys Val
    1685                 1690                 1695

Asn Glu  Arg Gly Glu Tyr Phe  Trp Glu Lys Arg Val  Thr Gly Cys
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Pro Pro  Phe Asp Glu His Lys  Cys Leu Ala Glu Gly  Gly Lys Ile
    1715                 1720                 1725

Met Lys  Ile Pro Gly Thr Cys  Cys Asp Thr Cys Glu  Glu Pro Glu
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Cys Asn  Asp Ile Thr Ala Arg  Leu Gln Tyr Val Lys  Val Gly Ser
    1745                 1750                 1755

Cys Lys  Ser Glu Val Glu Val  Asp Ile His Tyr Cys  Gln Gly Lys
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Cys Ala  Ser Lys Ala Met Tyr  Ser Ile Asp Ile Asn  Asp Val Gln
    1775                 1780                 1785

Asp Gln  Cys Ser Cys Cys Ser  Pro Thr Arg Thr Glu  Pro Met Gln
    1790                 1795                 1800

Val Ala  Leu His Cys Thr Asn  Gly Ser Val Val Tyr  His Glu Val
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Leu Asn  Ala Met Glu Cys Lys  Cys Ser Pro Arg Lys  Cys Ser Lys
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<210> SEQ ID NO 5
<211> LENGTH: 6075
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

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ggagacatcc aggtggtgcc cattggagtg ggccctaatg ccaacgtgca ggagctggag   4920
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gctcctgacc tggtgctgca gaggtgctgc tccggagagg ggctgcagat ccccaccctc   5040
tcccctgcac ctgactgcag ccagcccctg gacgtgatcc ttctcctgga tggctcctcc   5100
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gccaatatag ggcctcgtct cactcaggtg tcagtgctgc agtatggaag catcaccacc   5220

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atgcagcggg agggaggccc cagccaaatc ggggatgcct tgggctttgc tgtgcgatac   5340

ttgacttcag aaatgcatgg tgccaggccg ggagcctcaa aggcggtggt catcctggtc   5400

acggacgtct ctgtggattc agtggatgca gcagctgatg ccgccaggtc caacagagtg   5460

acagtgttcc ctattggaat tggagatcgc tacgatgcag cccagctacg gatcttggca   5520

ggcccagcag gcgactccaa cgtggtgaag ctccagcgaa tcgaagacct ccctaccatg   5580

gtcaccttgg gcaattcctt cctccacaaa ctgtgctctc gtgatgagac gctccaggat   5640

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aaaattccag gcacctgctg tgacacatgt gaggagcctg agtgcaacga catcactgcc   5820

aggctgcagt atgtcaaggt gggaagctgt aagtctgaag tagaggtgga tatccactac   5880

tgccagggca aatgtgccag caaagccatg tactccattg acatcaacga tgtgcaggac   5940

cagtgctcct gctgctctcc gacacggacg gagcccatgc aggtggccct gcactgcacc   6000

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<210> SEQ ID NO 6
<211> LENGTH: 2023
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile
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Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr
            20                  25                  30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly
        35                  40                  45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly
    50                  55                  60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys
65                  70                  75                  80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu
                85                  90                  95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro
            100                 105                 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys
        115                 120                 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly
    130                 135                 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly
145                 150                 155                 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln
                165                 170                 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala
            180                 185                 190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser
        195                 200                 205
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Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln
    210                 215                 220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu
225                 230                 235                 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu
                245                 250                 255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala
            260                 265                 270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His
        275                 280                 285

Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys
    290                 295                 300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met
305                 310                 315                 320

Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro Glu Gly Gln Leu
                325                 330                 335

Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His
            340                 345                 350

Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn
        355                 360                 365

Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys
    370                 375                 380

Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp
385                 390                 395                 400

Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg
                405                 410                 415

Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys
            420                 425                 430

Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu
        435                 440                 445

Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val
    450                 455                 460

Ala Met Asp Gly Gln Asp Val Gln Leu Pro Leu Leu Lys Gly Asp Leu
465                 470                 475                 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu
                485                 490                 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu
            500                 505                 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn
        515                 520                 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro
    530                 535                 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln
545                 550                 555                 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met
                565                 570                 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe
            580                 585                 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys
        595                 600                 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly
    610                 615                 620
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Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val
625                 630                 635                 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln
                645                 650                 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu
            660                 665                 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe
        675                 680                 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys
    690                 695                 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp
705                 710                 715                 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met
                725                 730                 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val
            740                 745                 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg
        755                 760                 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu
    770                 775                 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met
785                 790                 795                 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg
                805                 810                 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln
            820                 825                 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr
        835                 840                 845

Cys Val Cys Gln Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp
    850                 855                 860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly
865                 870                 875                 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp
                885                 890                 895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys
            900                 905                 910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu
        915                 920                 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys
    930                 935                 940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg
945                 950                 955                 960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg
                965                 970                 975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val
            980                 985                 990

Cys Gly Leu Cys Gly Asn Phe Asp  Gly Ile Gln Asn Asn  Asp Leu Thr
        995                 1000                 1005

Ser Ser  Asn Leu Gln Val Glu  Glu Asp Pro Val Asp  Phe Gly Asn
    1010                 1015                 1020

Ser Trp  Lys Val Ser Ser Gln  Cys Ala Asp Thr Arg  Lys Val Pro
    1025                 1030                 1035
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Leu Asp  Ser Ser Pro Ala Thr  Cys His Asn Asn Ile  Met Lys Gln
    1040                 1045                 1050

Thr Met  Val Asp Ser Ser Cys  Arg Ile Leu Thr Ser  Asp Val Phe
    1055                 1060                 1065

Gln Asp  Cys Asn Lys Leu Val  Asp Pro Glu Pro Tyr  Leu Asp Val
    1070                 1075                 1080

Cys Ile  Tyr Asp Thr Cys Ser  Cys Glu Ser Ile Gly  Asp Cys Ala
    1085                 1090                 1095

Cys Phe  Cys Asp Thr Ile Ala  Ala Tyr Ala His Val  Cys Ala Gln
    1100                 1105                 1110

His Gly  Lys Val Val Thr Trp  Arg Thr Ala Thr Leu  Cys Pro Gln
    1115                 1120                 1125

Ser Cys  Glu Glu Arg Asn Leu  Arg Glu Asn Gly Tyr  Glu Cys Glu
    1130                 1135                 1140

Trp Arg  Tyr Asn Ser Cys Ala  Pro Ala Cys Gln Val  Thr Cys Gln
    1145                 1150                 1155

His Pro  Glu Pro Leu Ala Cys  Pro Val Gln Cys Val  Glu Gly Cys
    1160                 1165                 1170

His Ala  His Cys Pro Pro Gly  Lys Ile Leu Asp Glu  Leu Leu Gln
    1175                 1180                 1185

Thr Cys  Val Asp Pro Glu Asp  Cys Pro Val Cys Glu  Val Ala Gly
    1190                 1195                 1200

Arg Arg  Phe Ala Ser Gly Lys  Lys Val Thr Leu Asn  Pro Ser Asp
    1205                 1210                 1215

Pro Glu  His Cys Gln Ile Cys  His Cys Asp Val Val  Asn Leu Thr
    1220                 1225                 1230

Cys Glu  Ala Cys Gln Glu Pro  Gly Gly Leu Val Val  Pro Pro Thr
    1235                 1240                 1245

Asp Ala  Pro Val Ser Pro Thr  Thr Leu Tyr Val Glu  Asp Ile Ser
    1250                 1255                 1260

Glu Pro  Pro Leu His Asp Phe  Tyr Cys Ser Arg Leu  Leu Asp Leu
    1265                 1270                 1275

Val Phe  Leu Leu Asp Gly Ser  Ser Arg Leu Ser Glu  Ala Glu Phe
    1280                 1285                 1290

Glu Val  Leu Lys Ala Phe Val  Val Asp Met Met Glu  Arg Leu Arg
    1295                 1300                 1305

Ile Ser  Gln Lys Trp Val Arg  Val Ala Val Val Glu  Tyr His Asp
    1310                 1315                 1320

Gly Ser  His Ala Tyr Ile Gly  Leu Lys Asp Arg Lys  Arg Pro Ser
    1325                 1330                 1335

Glu Leu  Arg Arg Ile Ala Ser  Gln Val Lys Tyr Ala  Gly Ser Gln
    1340                 1345                 1350

Val Ala  Ser Thr Ser Glu Val  Leu Lys Tyr Thr Leu  Phe Gln Ile
    1355                 1360                 1365

Phe Ser  Lys Ile Asp Arg Pro  Glu Ala Ser Arg Ile  Thr Leu Leu
    1370                 1375                 1380

Leu Met  Ala Ser Gln Glu Pro  Gln Arg Met Ser Arg  Asn Phe Val
    1385                 1390                 1395

Arg Tyr  Val Gln Gly Leu Lys  Lys Lys Lys Val Ile  Val Ile Pro
    1400                 1405                 1410

Val Gly  Ile Gly Pro His Ala  Asn Leu Lys Gln Ile  Arg Leu Ile
    1415                 1420                 1425
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Glu Lys  Gln Ala Pro Glu Asn  Lys Ala Phe Val Leu  Ser Ser Val
    1430                 1435                 1440

Asp Glu  Leu Glu Gln Gln Arg  Asp Glu Ile Val Ser  Tyr Leu Cys
    1445                 1450                 1455

Asp Leu  Ala Pro Glu Ala Pro  Pro Pro Thr Leu Pro  Pro Asp Met
    1460                 1465                 1470

Ala Gln  Val Thr Val Gly Pro  Gly Leu Leu Gly Val  Ser Thr Leu
    1475                 1480                 1485

Gly Pro  Lys Arg Asn Ser Met  Val Leu Asp Val Ala  Phe Val Leu
    1490                 1495                 1500

Glu Gly  Ser Asp Lys Ile Gly  Glu Ala Asp Phe Asn  Arg Ser Lys
    1505                 1510                 1515

Glu Phe  Met Glu Glu Val Ile  Gln Arg Met Asp Val  Gly Gln Asp
    1520                 1525                 1530

Ser Ile  His Val Thr Val Leu  Gln Tyr Ser Tyr Met  Val Thr Val
    1535                 1540                 1545

Glu Tyr  Pro Phe Ser Glu Ala  Gln Ser Lys Gly Asp  Ile Leu Gln
    1550                 1555                 1560

Arg Val  Arg Glu Ile Arg Tyr  Gln Gly Gly Asn Arg  Thr Asn Thr
    1565                 1570                 1575

Gly Leu  Ala Leu Arg Tyr Leu  Ser Asp His Ser Phe  Leu Val Ser
    1580                 1585                 1590

Gln Gly  Asp Arg Glu Gln Ala  Pro Asn Leu Val Tyr  Met Val Thr
    1595                 1600                 1605

Gly Asn  Pro Ala Ser Asp Glu  Ile Lys Arg Leu Pro  Gly Asp Ile
    1610                 1615                 1620

Gln Val  Val Pro Ile Gly Val  Gly Pro Asn Ala Asn  Val Gln Glu
    1625                 1630                 1635

Leu Glu  Arg Ile Gly Trp Pro  Asn Ala Pro Ile Leu  Ile Gln Asp
    1640                 1645                 1650

Phe Glu  Thr Leu Pro Arg Glu  Ala Pro Asp Leu Val  Leu Gln Arg
    1655                 1660                 1665

Cys Cys  Ser Gly Glu Gly Leu  Gln Ile Pro Thr Leu  Ser Pro Ala
    1670                 1675                 1680

Pro Asp  Cys Ser Gln Pro Leu  Asp Val Ile Leu Leu  Leu Asp Gly
    1685                 1690                 1695

Ser Ser  Ser Phe Pro Ala Ser  Tyr Phe Asp Glu Met  Lys Ser Phe
    1700                 1705                 1710

Ala Lys  Ala Phe Ile Ser Lys  Ala Asn Ile Gly Pro  Arg Leu Thr
    1715                 1720                 1725

Gln Val  Ser Val Leu Gln Tyr  Gly Ser Ile Thr Thr  Ile Asp Val
    1730                 1735                 1740

Pro Trp  Asn Val Val Pro Glu  Lys Ala His Leu Leu  Ser Leu Val
    1745                 1750                 1755

Asp Val  Gln Arg Glu Gly Gly  Pro Ser Gln Ile Gly  Asp Ala Leu
    1760                 1765                 1770

Gly Phe  Ala Val Arg Tyr Leu  Thr Ser Glu Met His  Gly Ala Arg
    1775                 1780                 1785

Pro Gly  Ala Ser Lys Ala Val  Val Ile Leu Val Thr  Asp Val Ser
    1790                 1795                 1800

Val Asp  Ser Val Asp Ala Ala  Ala Asp Ala Ala Arg  Ser Asn Arg
    1805                 1810                 1815
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Val Thr  Val Phe Pro Ile Gly  Ile Gly Asp Arg Tyr  Asp Ala Ala
    1820                 1825                 1830

Gln Leu  Arg Ile Leu Ala Gly  Pro Ala Gly Asp Ser  Asn Val Val
    1835                 1840                 1845

Lys Leu  Gln Arg Ile Glu Asp  Leu Pro Thr Met Val  Thr Leu Gly
    1850                 1855                 1860

Asn Ser  Phe Leu His Lys Leu  Cys Ser Arg Asp Glu  Thr Leu Gln
    1865                 1870                 1875

Asp Gly  Cys Asp Thr His Phe  Cys Lys Val Asn Glu  Arg Gly Glu
    1880                 1885                 1890

Tyr Phe  Trp Glu Lys Arg Val  Thr Gly Cys Pro Pro  Phe Asp Glu
    1895                 1900                 1905

His Lys  Cys Leu Ala Glu Gly  Gly Lys Ile Met Lys  Ile Pro Gly
    1910                 1915                 1920

Thr Cys  Cys Asp Thr Cys Glu  Glu Pro Glu Cys Asn  Asp Ile Thr
    1925                 1930                 1935

Ala Arg  Leu Gln Tyr Val Lys  Val Gly Ser Cys Lys  Ser Glu Val
    1940                 1945                 1950

Glu Val  Asp Ile His Tyr Cys  Gln Gly Lys Cys Ala  Ser Lys Ala
    1955                 1960                 1965

Met Tyr  Ser Ile Asp Ile Asn  Asp Val Gln Asp Gln  Cys Ser Cys
    1970                 1975                 1980

Cys Ser  Pro Thr Arg Thr Glu  Pro Met Gln Val Ala  Leu His Cys
    1985                 1990                 1995

Thr Asn  Gly Ser Val Val Tyr  His Glu Val Leu Asn  Ala Met Glu
    2000                 2005                 2010

Cys Lys  Cys Ser Pro Arg Lys  Cys Ser Lys
    2015                 2020
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<210> SEQ ID NO 7
<211> LENGTH: 4329
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

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ggtgagctgc ctgtggacgc aagatttcct cctagagtgc caaaatcttt tccattcaac     180

acctcagtcg tgtacaaaaa gactctgttt gtagaattca cggatcacct tttcaacatc     240

gctaagccaa ggccaccctg gatgggtctg ctaggtccta ccatccaggc tgaggtttat     300

gatacagtgg tcattacact taagaacatg gcttcccatc ctgtcagtct tcatgctgtt     360

ggtgtatcct actggaaagc ttctgaggga gctgaatatg atgatcagac cagtcaaagg     420

gagaaagaag atgataaagt cttccctggt ggaagccata catatgtctg gcaggtcctg     480

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gggagtctgg ccaaggaaaa gacacagacc ttgcacaaat ttatactact tttgctgta      660

tttgatgaag ggaaaagttg gcactcagaa acaaagaact ccttgatgca ggatagggat     720

gctgcatctg ctcgggcctg gcctaaaatg cacacagtca atggttatgt aaacaggtct     780

ctgccaggtc tgattggatg ccacaggaaa tcagtctatt ggcatgtgat tggaatgggc     840

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tgggtacatt acattgctgc tgaagaggag gactgggact atgctccctt agtcctcgcc   1260
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ctgcttacca gcatgtatgt gaaggagttc ctcatctcca gcagtcaaga tggccatcag   4140

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acacctgtgg tgaactctct agacccaccg ttactgactc gctaccttcg aattcacccc   4260

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<210> SEQ ID NO 8
<211> LENGTH: 1442
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
1               5                   10                  15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
            20                  25                  30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
        35                  40                  45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
    50                  55                  60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65                  70                  75                  80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
                85                  90                  95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
            100                 105                 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
        115                 120                 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
    130                 135                 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145                 150                 155                 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
                165                 170                 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
            180                 185                 190
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Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
        195                 200                 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
    210                 215                 220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
225                 230                 235                 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
                245                 250                 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
            260                 265                 270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
        275                 280                 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
    290                 295                 300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
305                 310                 315                 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
                325                 330                 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
            340                 345                 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
        355                 360                 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
    370                 375                 380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385                 390                 395                 400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
                405                 410                 415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
            420                 425                 430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
        435                 440                 445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
    450                 455                 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465                 470                 475                 480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
                485                 490                 495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
            500                 505                 510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
        515                 520                 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
    530                 535                 540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545                 550                 555                 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
                565                 570                 575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
            580                 585                 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
        595                 600                 605
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Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
    610                 615                 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                 630                 635                 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
                645                 650                 655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
            660                 665                 670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
        675                 680                 685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
    690                 695                 700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
705                 710                 715                 720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
                725                 730                 735

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
            740                 745                 750

Asn Asn Ala Ile Glu Pro Arg Ile Thr Arg Thr Thr Leu Gln Ser Asp
        755                 760                 765

Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys
    770                 775                 780

Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser
785                 790                 795                 800

Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu
                805                 810                 815

Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala
            820                 825                 830

Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe
        835                 840                 845

Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu
    850                 855                 860

His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn
865                 870                 875                 880

Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr
                885                 890                 895

Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro
            900                 905                 910

Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys
        915                 920                 925

Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala
    930                 935                 940

Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly
945                 950                 955                 960

Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala
                965                 970                 975

His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile
            980                 985                 990

Phe Asp Glu Thr Lys Ser Trp Tyr  Phe Thr Glu Asn Met  Glu Arg Asn
        995                 1000                1005

Cys Arg  Ala Pro Cys Asn Ile  Gln Met Glu Asp Pro  Thr Phe Lys
    1010                1015                1020
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```
Glu Asn  Tyr Arg Phe His Ala  Ile Asn Gly Tyr Ile  Met Asp Thr
    1025                 1030                 1035

Leu Pro  Gly Leu Val Met Ala  Gln Asp Gln Arg Ile  Arg Trp Tyr
    1040                 1045                 1050

Leu Leu  Ser Met Gly Ser Asn  Glu Asn Ile His Ser  Ile His Phe
    1055                 1060                 1065

Ser Gly  His Val Phe Thr Val  Arg Lys Lys Glu Glu  Tyr Lys Met
    1070                 1075                 1080

Ala Leu  Tyr Asn Leu Tyr Pro  Gly Val Phe Glu Thr  Val Glu Met
    1085                 1090                 1095

Leu Pro  Ser Lys Ala Gly Ile  Trp Arg Val Glu Cys  Leu Ile Gly
    1100                 1105                 1110

Glu His  Leu His Ala Gly Met  Ser Thr Leu Phe Leu  Val Tyr Ser
    1115                 1120                 1125

Asn Lys  Cys Gln Thr Pro Leu  Gly Met Ala Ser Gly  His Ile Arg
    1130                 1135                 1140

Asp Phe  Gln Ile Thr Ala Ser  Gly Gln Tyr Gly Gln  Trp Ala Pro
    1145                 1150                 1155

Lys Leu  Ala Arg Leu His Tyr  Ser Gly Ser Ile Asn  Ala Trp Ser
    1160                 1165                 1170

Thr Lys  Glu Pro Phe Ser Trp  Ile Lys Val Asp Leu  Leu Ala Pro
    1175                 1180                 1185

Met Ile  Ile His Gly Ile Lys  Thr Gln Gly Ala Arg  Gln Lys Phe
    1190                 1195                 1200

Ser Ser  Leu Tyr Ile Ser Gln  Phe Ile Ile Met Tyr  Ser Leu Asp
    1205                 1210                 1215

Gly Lys  Lys Trp Gln Thr Tyr  Arg Gly Asn Ser Thr  Gly Thr Leu
    1220                 1225                 1230

Met Val  Phe Phe Gly Asn Val  Asp Ser Ser Gly Ile  Lys His Asn
    1235                 1240                 1245

Ile Phe  Asn Pro Pro Ile Ile  Ala Arg Tyr Ile Arg  Leu His Pro
    1250                 1255                 1260

Thr His  Tyr Ser Ile Arg Ser  Thr Leu Arg Met Glu  Trp Met Gly
    1265                 1270                 1275

Cys Asp  Leu Asn Ser Cys Ser  Met Pro Leu Gly Met  Glu Ser Lys
    1280                 1285                 1290

Ala Ile  Ser Asp Ala Gln Ile  Thr Ala Ser Ser Tyr  Phe Thr Asn
    1295                 1300                 1305

Met Phe  Ala Thr Trp Ser Pro  Ser Lys Ala Arg Leu  His Leu Gln
    1310                 1315                 1320

Gly Arg  Ser Asn Ala Trp Arg  Pro Gln Val Asn Asn  Pro Lys Glu
    1325                 1330                 1335

Trp Leu  Gln Val Asp Phe Gln  Lys Thr Met Lys Val  Thr Gly Val
    1340                 1345                 1350

Thr Thr  Gln Gly Val Lys Ser  Leu Leu Thr Ser Met  Tyr Val Lys
    1355                 1360                 1365

Glu Phe  Leu Ile Ser Ser Ser  Gln Asp Gly His Gln  Trp Thr Leu
    1370                 1375                 1380

Phe Phe  Gln Asn Gly Lys Val  Lys Val Phe Gln Gly  Asn Gln Asp
    1385                 1390                 1395

Ser Phe  Thr Pro Val Val Asn  Ser Leu Asp Pro Pro  Leu Leu Thr
    1400                 1405                 1410
```

```
Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala
    1415                1420                1425

Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
    1430                1435                1440


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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 9

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<400> SEQUENCE: 10

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<210> SEQ ID NO 11
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<212> TYPE: DNA
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<400> SEQUENCE: 11

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<210> SEQ ID NO 13
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agagcacagt ttgtggag                                                    18


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<212> TYPE: DNA
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ttttctggtg tcagcacact g                                                21


<210> SEQ ID NO 19
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<212> TYPE: DNA
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caggtgcagg ggagagg                                                     17
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What is claimed is:

1. A mutant von Willebrand factor (vWF32) having the amino acid sequence of SEQ ID NO: 6 in which exons 33-48 of vWF are deleted.

2. A mutant vWF32 gene having a base sequence encoding for a protein having the amino acid sequence of SEQ ID NO: 6.

3. The mutant vWF32 gene as set forth in claim **2**, wherein the mutant vWF32 gene has the base sequence of SEQ ID NO: 5.

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